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ARTICLES

EFFECT OF CERTAIN CHANGES IN TESTING TECHNIQUE ON MORTALITY OF MOSQUITO LARVAE [1]

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The use of mosquito larvae in laboratory tests to compare toxicity of chemicals and to assay resistance has focused attention on the variation in mortality obtained with different testing techniques. The available data do not completely account for this variation. Kruse *et al.* (1952) concluded from tests against *Anopheles quadrimaculatus* Say that the number of larvae, the volume of liquid per test, the mass-surface area per larva, the formulation, and the type of surface of the container will influence the results obtained with DDT. Hawkins and Kearns (1956) reported on the ionic environment and zeta potential in relation to suspension stability of DDT. Hawkins (1956) reported that the volume of ethanol solvent used to prepare suspensions of DDT in water affected the mortality of *quadrimaculatus* in tests in which the solvent content was varied by diluting a stock suspension rather than preparing new suspensions at each concentration. In tests with acetone-water suspensions or solutions of three radioactive insecticides, Schmidt and Weidhaas (1958) showed that increasing the number of *quadrimaculatus* larvae per test decreased the mortality and the absorbed dosage of the insecticide in tests with DDT, but not with Bayer 21/199 or Am. Cyanamid 12880. They concluded that the importance of this factor varies with the chemical used and the percentage removed by the larvae.

Experiments were conducted at Orlando, Fla., to determine the effect of the more obvious variables on the mortality of laboratory-reared larvae of *quadrimaculatus* and/or *Aedes taeniorhynchus* (Wied.) when exposed to acetone-water suspensions of DDT. The variables studied with both species were susceptibility of different instars and volume of test suspension. Those studied with *quadrimaculatus* only were quantity of acetone per test container, method of preparing DDT suspensions, and feeding or starving larvae prior to test.

TEST METHODS. The variables were introduced one at a time into the standard test method used at Orlando, and comparisons were made with that method. In the Orlando method, with laboratory-reared mosquitoes, larvae are removed from the rearing pans on the day of testing after they have fed 3 to 4 hours on fresh food and are pooled in clean water. Twenty-five early fourth instar larvae are placed in 25 ml. of distilled water in a 50-ml. beaker. Suspensions are prepared by pipetting an appropriate amount of an acetone solution of the material to be tested (in these tests DDT) into 225 ml. of distilled water in a glass jar and stirring. The water containing the larvae is poured into the test suspension; this is done 2 to 3 hours after the removal of the larvae from the rearing pans and 4 hours after preparation of the test suspension.

In the tests here reported duplicate jars or pans of five or six concentrations of DDT ranging from 0.0010 to 0.050 p.p.m. for *quadrimaculatus* and 0.0050 to 0.10 p.p.m. for *taeniorhynchus* were used. Each test was replicated three times. There would have been less variability in mortality results if all tests had been run

[1] From the Papers and Proceedings of the 14th Annual Meeting of the AMCA, Washington, D. C., February 23–26, 1958.

[2] The authors wish to express their gratitude to H. R. Ford who did the laboratory work.

TABLE 1.—Susceptibility of different instars of *Anopheles quadrimaculatus* and *Aedes taeniorhynchus* larvae to acetone-water suspensions of DDT

Instar	24-hour exposure			48-hour exposure		
	LC–50 (p.p.m.)	LC–90 (p.p.m.)	Slope	LC–50 (p.p.m.)	LC–90 (p.p.m.)	Slope
			Anopheles quadrimaculatus			
Second	0.0021±.0005	0.0048±.0006	3.58±.55	—[a]	—[a]	—[a]
Third	.0033±.0003	.0068±.0009	4.35±.45	0.0022±.0002	0.0049±.0003	3.58±.19
Fourth	.0052±.0003	.0110±.0010	3.98±.33	.0042±.0005	.0110±.0009	3.33±.24
			Aedes taeniorhynchus			
Second	0.0027±.0002	0.0055±.0003	4.19±.25	0.0026±.0003	0.0051±.0004	4.35±.36
Third	.025 ±.0029	.059 ±.0090	3.61±.24	.0084±.0005	.024 ±.0035	3.16±.34
Fourth	.047 ±.005	—[b]	1.33±.33	.036 ±.0085	—[b]	1.45±.26

[a] Percent mortality too high for analysis.
[b] Percent mortality too low to obtain a valid LC–90.

TABLE 2.—Toxicity of acetone-water suspensions of DDT to mosquito larvae in different test volumes and containers. (250 ml. in glass jars, 1,000 ml. in enamel pans.)

Test volume (milliliters)	24-hour exposure			48-hour exposure		
	LC–50 (p.p.m.)	LC–90 (p.p.m.)	Slope	LC–50 (p.p.m.)	LC–90 (p.p.m.)	Slope
			Anopheles quadrimaculatus			
250	0.0079±.0008	0.015±.002	4.43±.19	0.0046±.0007	0.010±.0019	3.90±.44
1,000	.0088±.0013	.019±.002	4.16±.02	.0063±.0009	.016±.0018	3.28±.31
			Aedes taeniorhynchus			
250	0.038±.004	0.163±.028	2.20±.25	0.013±.006	0.040±.010	2.85±.29
1,000	.021±.004	.076±.012	2.35±.23	.014±.002	.035±.004	3.42±.32

on one batch of larvae, but these replicates were run on different days with different batches to approximate the variability that would be inherent in the use of this method for resistance studies. Mortality readings were taken at 24 and 48 hours, and the LC–50, LC–90, and slope were calculated. Since mortalities were sometimes too low at 24 hours or too high at 48 hours, the calculations were based on three to five significant concentrations.

In the first series of tests this method was used to determine the susceptibility of second, third, and fourth instar larvae. A second series was run to compare the mortality obtained in 1 liter of suspension in enamel pans with that in 250 ml. in glass jars. The pans were 8½ inches in diameter and the water was 1⅛ inches deep, whereas the jars were 3 inches in diameter and the water was 2⅛ inches deep. In a third series larvae were exposed by the standard method except that sufficient acetone was added to each jar to give a total of 1, 2, and 5 ml. of acetone. Since some acetone may have evaporated during the 4 hours between preparation of the suspensions and introduction of the larvae, this test was repeated and the larvae were introduced immediately after preparation of the suspensions. A comparison also was made of the effect of adding various concentrations of DDT in a uniform volume of acetone (1.25 ml.) and of adding the same concentrations in various volumes of acetone (0.31 to 1.25 ml.) as is done in the standard method. Finally, the susceptibility of larvae fed up to the time of testing was compared with that of others starved for 4 hours.

SUSCEPTIBILITY OF DIFFERENT INSTARS. With both species the susceptibility to DDT decreased with the age of the larvae, as shown in table 1. With *quadrimaculatus* the LC–50 and LC–90 increased 1.6 and 1.4 times from the second to the third instar after 24 hours and 1.6 times from the third to the fourth instar after 24 hours and 1.9 to 2.2 times after 48 hours. With *taeniorhynchus* the respective increases from the second to the third instar were 9.3 to 10.7 times at 24 hours and 3.2 to 4.7 times at 48 hours, and from the third to the fourth instars 1.9 times at 24 hours and 4.3 times at 48 hours. The *taeniorhynchus* larvae, which were from a colony resistant to DDT, showed a much greater increase between the second and fourth instars than did *quadrimaculatus*. After 24 hours second instar larvae of both species were approximately equal in susceptibility, whereas the LC–50 of fourth instar *taeniorhynchus* was about nine times that for fourth instar *quadrimaculatus*. With *quadrimaculatus* the slopes of the regression lines do not show significant differences between instars; however, with *taeniorhynchus* they are significantly flatter for each succeeding instar.

VOLUME OF TEST SUSPENSION. The results with 250 ml. of suspension in glass jars compared with those with 1 liter in enamel pans are given in table 2. With *quadrimaculatus* the LC–50 and LC–90 were not significantly different at 24 hours, but at 48 hours they were significantly lower with 250 ml. With *taeniorhynchus* the LC–50 and LC–90 were significantly lower at 24 hours with 1 liter, but at 48 hours there were no significant differences. Since the total available DDT per larva was four times as great in the tests with 1 liter as in those with 250 ml., it can be concluded that the total available DDT was not the single factor controlling mortality.

QUANTITY OF ACETONE. The results of tests in which the content of acetone was varied are given in table 3. There was no significant difference in mortality in suspensions containing 1, 2, or 5 ml. of acetone when larvae were added immediately after the acetone and when they were added after 4 hours the only significant difference occurred in the LC–90 with 5 ml. of acetone.

METHOD OF PREPARING DDT SUSPENSIONS. The results of tests to compare two methods of preparing a range of DDT suspensions are given in table 4. When the concentration of DDT in the acetone was kept constant, and different volumes

TABLE 3.—Effect of different volumes of acetone on the toxicity of DDT to fourth-instar larvae of *Anopheles quadrimaculatus*

Volume of acetone (ml.)	24-hour exposure			48-hour exposure		
	LC–50 (p.p.m.)	LC–90 (p.p.m.)	Slope	LC–50 (p.p.m.)	LC–90 (p.p.m.)	Slope
			Larvae introduced 4 hours later			
1	0.0052±.0007	0.013 ±.0030	3.67±.40	0.0029±.0004	0.011 ±.0011	2.69± .47
2	.0052±.0005	.010 ±.0010	4.71±.45	.0036±.0007	.0092±.0037	3.34± .43
5	.0044±.0004	.0075±.0010	5.86±.42	.0027±.0003	.0066±.0007	3.37± .27
			Larvae introduced immediately			
1	.0055±.0002	.011 ±.0013	4.58±.81	.0029±.0006	.0089±.0011	2.56± .16
2	.0059±.0006	.012 ±.0018	4.54±.63	.0027±.0006	.0086±.0017	2.53± .13
5	.0049±.0005	.010 ±.0009	4.02±.08	.0030±.0012	.0066±.0019	3.79±1.28

TABLE 4.—Comparison of effects of two methods of preparing DDT suspensions on toxicity to fourth-instar *Anopheles quadrimaculatus* larvae

Method of preparation	24-hour exposure			48-hour exposure		
	LC–50 (p.p.m.)	LC–90 (p.p.m.)	Slope	LC–50 (p.p.m.)	LC–90 (p.p.m.)	Slope
Varying volume of acetone, constant concentration	0.0051±.0003	0.0098±.0010	4.76±.32	0.0034±.0003	0.0081±.0008	3.45±.20
Constant volume of acetone, varying concentration	.0067±.0008	.013 ±.002	4.60±.30	.0048±.0003	.010 ±.0012	3.96±.59

TABLE 5.—Toxicity of acetone-water suspensions of DDT to fourth-instar *Anopheles quadrimaculatus* larvae when they were starved for 4 hours or fed up to the time of testing

	24-hour exposure			48-hour exposure		
	LC–50 (p.p.m.)	LC–90 (p.p.m.)	Slope	LC–50 (p.p.m.)	LC–90 (p.p.m.)	Slope
Fed	0.0065±.0013	0.013±.0010	4.31±.24	0.0044±.0010	0.010±.0007	3.72±.55
Unfed	0.0065±.0008	0.012±.0010	4.38±.56	0.0047±.0005	0.011±.0010	3.74±.39

of acetone were added to the water, the LC–50 and LC–90 were not as high as when the volume of acetone was kept constant and the concentration varied. However, the slopes of the regression lines were not significantly different, and the LC–50 and LC–90 obtained at a constant volume were no higher than those in some other tests with varying volumes, such as those given in the first line of table 2. It is therefore concluded that when acetone is used as the solvent the two methods give equal results.

EFFECT OF FEEDING LARVAE. As shown in table 5, there was no significant difference in mortality between larvae fed up to the time of testing and those starved for 4 hours.

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THE STRATIFICATION OF MOSQUITOES

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INTRODUCTION. As part of studies on the bionomics of mosquitoes in southwestern Georgia, collections were made at six elevations from 3 feet to 50 feet in an effort to determine the extent of natural stratification among mosquito species.

Previous studies on the vertical distribution of mosquitoes have been made in this country (MacCreary, 1941; Gjullin *et al.*, 1950; Snow, 1955) and in other parts of the world (Davis, 1944; Bates, 1944; Haddow *et al.*, 1945 a and b, 1947, 1948, 1949; Garnham *et al.*, 1946; Kumm *et al.*, 1946, 1951; Galindo *et al.*, 1951), but in these investigations attractants were employed. Studies conducted in this area of Georgia on the stratification of mosquitoes at various times throughout the night as measured by light traps are reported elsewhere (Love *et al.*, manuscript). The present report gives the results of mosquito collections made without attractants.

MATERIALS AND METHODS. From May 1954 to July 1956 collections of mosquitoes were made one night each week with mechanical sweeping devices at elevations of 3-, 6-, 15-, 25-, 40-, and 50-foot elevations in a wooded area adjacent to a breed-

ing site. The mechanical nets, attached to a specially constructed tower, were rotated at approximately 25 r.p.m. from 6 p.m. until about 9 a.m. the following morning. A complete description of the sweep nets has been given previously (Love and Smith, 1957).

Only collections made on the 85 nights when all nets operated successfully were used in this analysis. After the numbers of mosquitoes collected had been totaled by species and listed in tabular form, the collections for those species which showed similar distribution characteristics were then totaled and plotted graphically to illustrate the differences in patterns of vertical distribution. To eliminate the abundance factor from the graphic presentation of the data, the maximum collections of all patterns were plotted on a common axis. The remaining points were then shown as percentages of these maxima.

RESULTS AND DISCUSSION. Ten species of mosquitoes were used in the analysis. Most of these were present regularly in the collections but some, particularly the *Culiseta* spp., were taken sporadically and in small numbers. The vertical distribution indicated for the *Culiseta* was distinctive enough, however, to justify inclusion. The numbers of specimens of each species collected at each elevation are shown in Table 1. Four types of distribution patterns were observed. Species showing similar distribution characteristics were grouped together and their collections totaled, as follows: pattern 1—*Anopheles crucians, A. quadrimaculatus,* and *Uranotaenia sapphirina;* pattern 2—*Aedes infirmatus, Ae. vexans,* and *Psorophora ferox;* pattern 3—*Culex restuans* and *C. (Melanoconion)* sp.; and pattern 4—*Culiseta inornata* and *Cu. melanura.* Too few representatives of other species were present in the collections to warrant use in this study.

These four types of distribution patterns are shown diagrammatically in Figure 1. Each pattern is typical for a particular genus or for ecologically related genera. The maximum numbers of *Anopheles* and *Uranotaenia* were collected at 6 feet, 51 percent of maximum at 3 feet, with a marked decrease at 15 feet continuing through higher elevations. The maximum numbers of *Aedes* and *Psorophora* were collected at 40 feet, with near maximum collections also taken at 25 feet and 3 feet. Fewer but still appreciable numbers were collected at 6 feet, 15 feet, and at the high 50-foot level. The greatest number of *Culex* was collected at 3 feet, the numbers decreasing at higher elevations, except for a secondary peak at 25 feet. *Culiseta* was collected most frequently at 3 feet, with collections at other elevations varying only from 64 to 73 percent of this maximum.

These distribution patterns indicate that *Anopheles* and *Uranotaenia* are predominantly low fliers, and only rarely get as high as 15 feet above the ground. The *Aedes* and *Psorophora* fly more uniformly at higher elevations, being rather evenly distributed throughout the vegetative canopy. *Culex* apparently flies at higher elevations than do *Anopheles* or *Uranotaenia* but not as high as *Aedes* or *Psorophora. Culiseta* is evenly distributed from 6 feet to 50 feet. The larger proportions of the collections taken at 3 feet probably indicate that most specimens of the genera studied rest during the day in low vegetation and are therefore subject to capture at the time nocturnal activity commences and the following morning when individuals are again seeking suitable diurnal resting places.

Although no attractant was used to collect the specimens for this study, undoubtedly there were motivating factors which determine the approximate elevation at which the greatest number of individuals was active. Bates (1949) listed factors believed to influence the vertical distribution of mosquitoes as gradients in temperature, humidity, and light; specific resistance to desiccation; search for oviposition sites; swarming and mating activities; and the location of their larval habitats. It appears that the activity of food seeking

TABLE 1.—Number of mosquitoes collected in mechanical sweep nets located from 3 feet to 50 feet above the ground, Baker County, Georgia, May 1954-July 1956

Pattern 1: *Anopheles* and *Uranotaenia*

Elevation (in feet)	*Anopheles crucians*	*Anopheles quadrimaculatus*	*Uranotaenia sapphirina*	Total
3	196	130	451	777
6	345	139	1,029	1,513
15	28	10	16	54
25	7	5	11	23
40	1	1	5	7
50	2	0	7	9
Total	579	285	1,519	2,383

Pattern 2: *Aedes* and *Psorophora*

Elevation (in feet)	*Aedes infirmatus*	*Aedes vexans*	*Psorophora ferox*	Total
3	144	890	183	1,217
6	48	526	98	672
15	24	743	62	829
25	32	1,166	47	1,245
40	66	1,132	72	1,270
50	61	928	69	1,058
Total	375	5,385	531	6,291

Pattern 3: *Culex*

Elevation (in feet)	*Culex restuans*	*Culex* (*Melanoconion*) sp.	Total
3	100	71	171
6	70	48	118
15	49	33	82
25	75	51	126
40	55	35	90
50	22	23	45
Total	371	261	632

Pattern 4: *Culiseta*

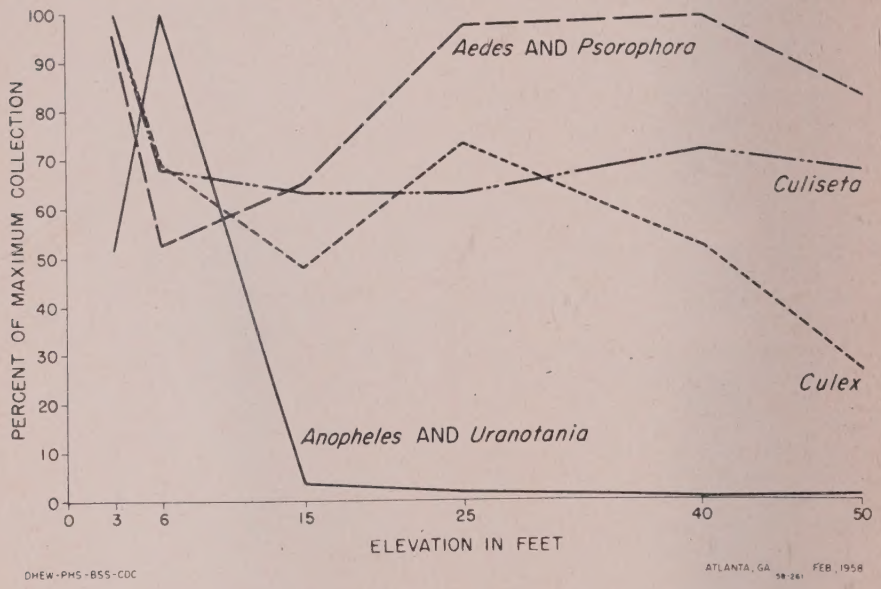
Elevation (in feet)	*Culiseta inornata*	*Culiseta melanura*	Total
3	6	16	22
6	3	12	15
15	5	9	14
25	3	11	14
40	4	12	16
50	6	9	15
Total	27	69	96

might well be added, since the availability of preferred hosts at a relatively constant height might indicate the level at which the majority of the females fly.

Results of thousands of precipitin tests have shown that *Anopheles* rarely feeds on hosts other than man or domestic mammals. *Uranotaenia* is thought to feed exclusively on cold blooded animals. All of these hosts occur near the ground in close association with the indicated distribution of these mosquito genera.

FIGURE 1

DISTRIBUTION PATTERNS FOR MOSQUITOES AS INDICATED BY MECHANICAL SWEEP NET COLLECTIONS, BAKER COUNTY, GEORGIA; MAY 1954-JULY 1956



The feeding habits of *Culex* are not so well known. In rural areas, greatest numbers of adult female *C. quinquefasciatus* and *C. erraticus* may be found associated with poultry. While chickens usually do not roost very far above the ground, this association may indicate the preference of these species for avian blood. The colonization of *C. quinquefasciatus* and the laboratory feeding of *C. restuans* is greatly facilitated by the use of birds as a source of blood. In the western United States *C. tarsalis* is the chief vector of encephalitis, the pathogen of which occurs in birds. Since this species transmits the infection to man, the indicated association is with both birds and man, with birds probably being the preferred hosts. A preferred avian host which consistently roosts in the tree canopy might require the *Culex* mosquitoes to fly at a higher elevation than *Anopheles* and *Uranotaenia.*

Several species of *Aedes* have been found naturally infected with encephalitis, although they are not thought to be important in transmitting infections to man. Many species of both *Aedes* and *Psorophora* have been shown capable of direct transmission of encephalitis virus in the laboratory (Ferguson, 1954).

If *Aedes* and *Psorophora* are predominantly higher fliers as indicated by their distribution pattern, they would be expected to feed primarily on birds. They could be very important then in maintaining encephalitis in the avian reservoir and be found infected with the virus, but still be of little importance as far as human infections are concerned. However, there is some evidence that *Ae. vexans* does not feed primarily on birds. Of approximately 35 freshly engorged specimens collected in the course of this study and tested to determine the source of the blood meal, not

one had fed on avian blood. Burroughs and Burroughs (1954) reported that 77 percent of 202 *Ae. vexans* collected in the upper Mississippi Valley had fed on domestic mammals as indicated by precipitin tests.

The only information concerning the feeding habits of *Culiseta* is the reported incrimination of *Cu. melanura* as the vector of encephalitis in domestic pheasants in New Jersey. Both *Cu. melanura* and *Cu. inornata* were fed successfully on birds in the laboratory.

The observations point out that in some cases there may be correlation between the vertical distribution of mosquitoes and their feeding preferences, but that much additional work on the subject is desirable.

Summary. Mosquito collections made with mechanical sweeping devices which utilized no attractant indicated that the aerial distribution of various genera conforms to different patterns. *Anopheles* and *Uranotaenia* rarely attained an altitude of 15 feet. *Aedes* and *Psorophora* were more active at higher elevations, and *Culex* occurred most frequently at intermediate elevations. *Culiseta* was evenly distributed from 6 feet to 50 feet. There are correlations between the indicated vertical distribution and known feeding preferences.

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SOME OBSERVATIONS ON THE MOSQUITO FAUNA OF THE OKEFENOKEE SWAMP

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In June and September, 1957, visits were made to the Okefenokee Swamp as part of an arthropod-borne encephalitis study being conducted by this laboratory.[2] Recently there have been clinical cases of eastern equine encephalomyelitis near Waycross, Georgia, just north of the Okefenokee Swamp, and isolation of the virus of EEE was reported from three arthropod pools: *Anopheles crucians, Aedes mitchellae,* and *Culicoides* spp., collected in south Georgia in 1956 (1).

The Okefenokee Swamp lies for most of its 420,000 acres in Georgia, with a smaller portion extending over the state line into Florida (Fig. 1). By Executive Order in 1937, 329,000 acres of the Georgia swamp were designated a National Wildlife Refuge and ever since, a successful effort has been made by the Fish and Wildlife Service of the Department of the Interior to prevent further destruction of its flora and fauna. During two periods in the last hundred years the Okefenokee Swamp has been invaded by large groups of people for various purposes. In the 1830's General Charles Floyd and his Dragoons put to rout the renegade Creeks (the Seminoles), under the leadership of Chief Billy Bowlegs (2). Floyd and Billys Islands in the interior of the swamp were named for the leaders of the two forces. From 1850 until the 1920's lumbering was the major attraction. In 1891, hoping to gain access to the magnificant stands of virgin cypress, slash pine, red bay, and black gum, there was an unsuccessful attempt to drain the swamp (3). Commercial and sport hunters found the Okefenokee Swamp wildlife abundant. Alligators, deer, bear, and wildcat were harvested in quantity before wildlife protection was instituted. Now, as a refuge, the Okefenokee Swamp is meticulously managed. No animal may be killed, no tree cut, or plant removed without specific permission of the Fish and Wildlife Service. Many areas are now inaccessible though visitors are invited to see as much of the swamp as can be navigated safely from three access areas; Camp Cornelia to the east near Folkston, Camp Stephen Foster to the west near Fargo, and Okefenokee Swamp Park to the north near Waycross.

Two rivers, the Suwanee and St. Marys, have headwaters in the swamp. The coffee-colored, tannic acid water flows gently, almost imperceptibly through the swamp to form these two rivers. Within the swamp there are islands with luxuriant growth and "prairies" where water lilies abound so thickly that the tangled root system will support foraging bears, which occasionally are seen. Through the prairies are apparent channels two to four feet wide, called boat runs. Actually these are the paths of flowing water and passage ways for alligators. Clumps of trees surrounded by bog peat to give the appearance of an island occur throughout the prairies and are known as "houses." Some of the larger houses will support the weight of a man, but the floating bog will sink and recover with each step giving rise to the Indian name, Ouaguaphenogaw, corrupted to Oke-fe-no-kee, meaning "trembling earth."

HABITATION AND DISEASE. More than thirty years ago, when the Hebard Cypress Company and Twin Tree Lumber Company were at peak lumbering activity within the Swamp, probably more than a thousand people inhabited the inner islands. On Billys Island in 1924 the census was "450 people, 105 cattle, 3 mules, 162

[1] Third U. S. Army Medical Laboratory, Fort McPherson, Georgia.

[2] This study is conducted with funds allocated by the Research and Development Division, Office of The Army Surgeon General.

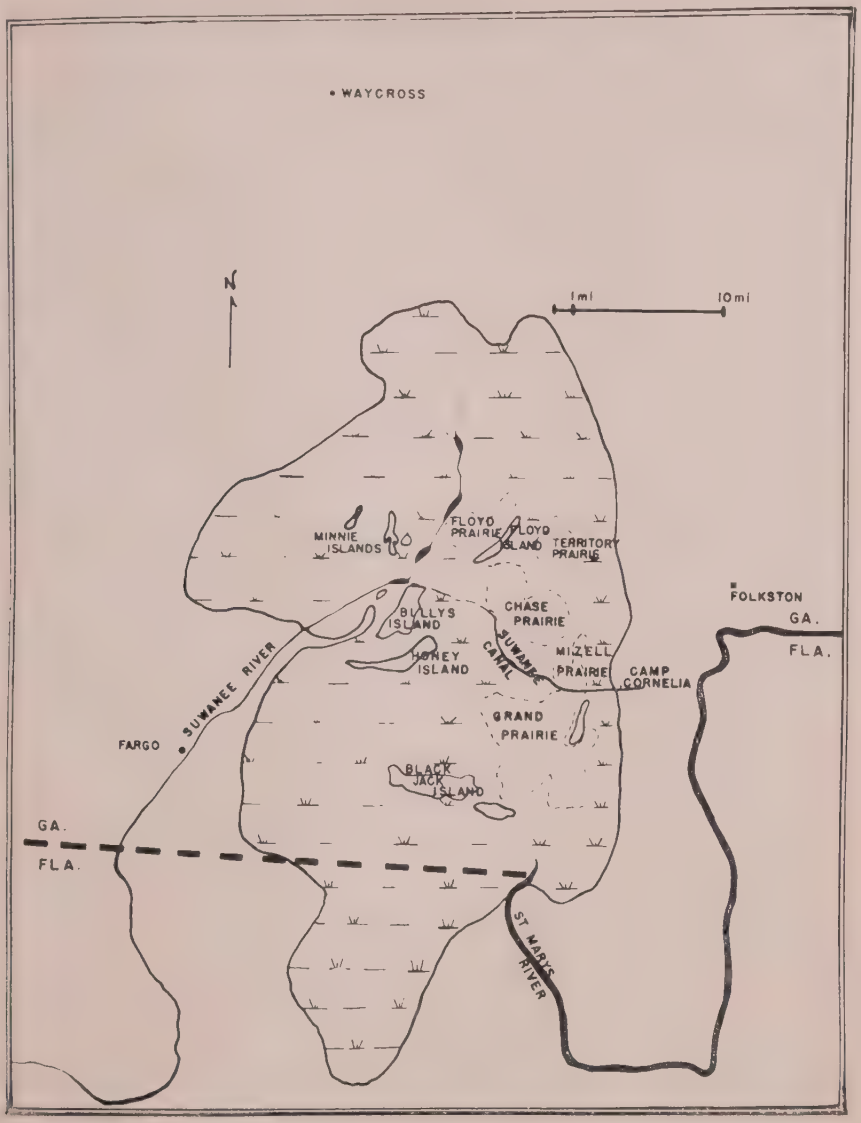

FIG. 1.—Okefenokee Swamp.

swine, and approximately 1,000 fowls" (4). When lumbering ceased to be profitable the companies dissolved, leaving behind some permanent residents. Gradually, the families moved or died out until today there are no families living within the swamp and no evidence of surviving domestic animals.

The Okefenokee always has been considered a healthy area. Even today the local people pridefully make this point. In the 1920's, when malaria was rampant in the southeastern United States, through the more accurate record-keeping period of the 1940's, the Okefenokee has been "mysteriously" free of malaria. According to legend, and some records, life in the Okefenokee seems to exert therapeutic as as well as prophylactic effect against clinical malaria. Early explorers traveled the St. Marys River to the swamp in order to obtain its fresh water for drinking purposes (3).[3] Many people employed by the lumbering companies of the 1920's came to the Swamp with histories of malaria and recrudescences, but no relapses occurred in these people after settling in the swamp. Of 933 histories and 347 blood films taken from persons residing within the swamp during the years of 1924–1925, not one was positive for malaria (4)

MOSQUITO SURVEYS. Mayne (4) reports the capture of a female *Anopheles quadrimaculatus* from a fringe area and its transport to the swamp where it was induced to lay eggs on water collected from the swamp. The female specimen was then destroyed. The eggs hatched in the swamp water and the larvae developed without incident for six days, at which time they were killed.

Fletcher (5) in a report on the pH tolerance of some anophelines points out that *Anopheles crucians* favors a more acid environment than *Anopheles quadrimaculatus*. During the 1957 survey pH readings were made by paper colorimetric method. The pH of the swamp water ranged between 4.8 and 5.5 by this method.

The results of the 1957 survey are presented in Table 1. As will be observed, *Anopheles quadrimaculatus* still was not taken in the swamp proper, though it was taken in limited numbers at Camp Cornelia during both visits. The genus *Aedes* was represented by five species on Floyd Island with *Aedes atlanticus-tormentor* being the most aggressive daytime biter. At Camp Cornelia two species of *Aedes* were encountered in June but none in September.

Evaluating the data in Table 1, one observes the low catch of *Anopheles crucians* and the relatively high catch of *Mansonia perturbans*. The portable light trap taken into the swamp proved inefficient, resulting in a reduced catch of *Anopheles crucians,* whereas *Mansonia perturbans,* a more active anthropophilic species, was taken readily in biting collections.

At Camp Cornelia in June 1957, combined light trap and biting collections produced a normal picture. In September 1957, however, the complete absence of *Aedes,* particularly *Aedes infirmatus* and *Aedes atlanticus-tormentor,* from both light trap and biting collections is an interesting void.

Larval collections were, in the main, unproductive. This also was true in the 1924–25 survey (4). Apparently most of the breeding takes place in semi-permanent to permanent pools of water well protected by undergrowth. Larval sampling in the prairies consistently was unproductive though it is quite likely that *Mansonia perturbans* thrives in this environment. Samplings from the pitcher plant, *Saracennia flava,* were negative. The most productive larval catches were made in the cypress swamps of Floyd Island, where *Culex territans, Anopheles crucians,* and *Culiseta melanura* were taken from a common source. *Aedes triseriatus* was taken from a magnolia tree hole.

[3] Bacteriological examination of Okefenokee Swamp water collected in November, 1957, was performed by the Third U. S. Army Medical Laboratory. The MPN coliforms/100 ml sample was 50.

TABLE 1.—Mosquito collections, Okefenokee Swamp, Georgia, June and September 1957

Species	3 June to 12 June 1957				Third Week, September 1957				
	Total Specimens	Floyd Island		Camp Cornelia	Total Specimens	Camp Stephen Foster		Camp Cornelia	Grand Total
		Adults	Larvae	Adults		Adults	Larvae	Adults	
Anopheles crucians	616	1	6	609	986	451	—	535	1602
Anopheles quadrimaculatus	2	—	—	2	16	—	—	16	18
Uranotaenia sapphirina	1	—	—	1	389	258	—	131	390
Culiseta melanura	102	—	7	95	104	91	—	13	206
Mansonia perturbans	35	22	—	13	57	37	—	20	92
Psorophora ciliata	26	—	—	26	1	—	—	1	27
Psorophora confinnis	12	—	—	12	11	—	—	11	23
Aedes atl.-tormentor	15	12	—	3	—	—	—	—	15
Aedes canadensis	1	1	—	—	—	—	—	—	1
Aedes infirmatus	26	26	—	—	—	—	—	—	26
Aedes mitchellae	2	—	—	2	—	—	—	—	2
Aedes triseriatus	4	2	2	—	—	—	—	—	4
Culex nigripalpus	—	—	—	—	1	1	—	—	1
Culex salinarius	1	—	—	1	6	6	—	—	7
Culex pilosus	—	—	—	—	54	—	54	—	54
Culex (Mel.) spp.	1	—	—	1	1	1	—	—	2
Culex territans	40	—	40	—	—	—	—	—	40
Total	884	64	55	765	1626	845	54	727	2510

Of further interest is the high percentage of *Culiseta melanura, Mansonia perturbans,* and *Anopheles crucians,* all natural vectors of EEE, on the fringe area of the swamp.

From these specimens a total of eighty-nine species-pure pools were formed and each was inoculated into groups of five, 21-day-old mice. In one, P-522,[4] an agent not yet identified is active. No passage has been successful, though replicates from the original material have produced the same lethal pattern.

Summary. The Okefenokee long has been an area of special interest, first as a hiding area for Creek Indians, then as an area for harvesting by lumberers and hunters, finally obtaining its present stature as a refuge for wildlife, a tourist attraction, and an area ideally suited for the student of nature.

The swamp is free of human malaria and the southeastern malaria vector, *Anopheles quadrimaculatus,* will not breed in its acid waters. Known vectors of EEE are present in abundance throughout the swamp, and the clinical disease is seen in the equine population of the bordering villages. Of eighty-nine arthropod pools collected during 1957 and inoculated into mice, none produced a recognizable disease.

[4] Third U. S. Army Medical Laboratory accession number. Ft. McPherson, Ga.

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A NATURAL HIBERNATION SITE OF THE MOSQUITO *CULEX TARSALIS* COQUILLETT IN THE COLUMBIA RIVER BASIN, WASHINGTON

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INTRODUCTION. Encephalitis viruses in temperate zones are admirably adapted to mosquitoes of the genus *Culex* whose species, insofar as known, overwinter in the adult stage. At this writing, neither a plausible or satisfactory mechanism for maintenance of western equine encephalitis (WEE) virus through severe winters in northern climates, nor for its reintroduction each spring, has been demonstrated. Until positively ruled out, attention must be focused on the vector mosquito, *Culex tarsalis,* as a potential reservoir.

The species thrives in cold climates where it survives the winter as fertile females. Ample experimental evidence has shown that *C. tarsalis,* once infected with WEE virus, remains infected for life. Thus, if sufficient numbers of infected, hence blood-fed, females can hibernate successfully, at least one mechanism for virus maintenance will have been revealed. As briefly reviewed below, considerable effort has been expended in searching for hibernation quarters of the species and in studying overwintering populations, but not until this report has a natural site been discovered.

REVIEW. Hammon *et al.* (1945) in the Yakima Valley, Washington, collected 18 *C. tarsalis* females among thousands of *C. pipiens* and other species in the winter. Collecting conditions are not given. Virus was not found.

Keener (1952) in western Nebraska recorded something less than 100 female *C. tarsalis* associated with *C. territans* in two outside food storage cellars from 13 January to 28 April 1951 when the last *C. tarsalis* was noted. The largest number seen in either cellar at any biweekly visit was 15 on 20 January and the lowest temperature in the cellars was 33° F. Only 18 mosquitoes were tested and no virus was found.

Blackmore and Winn (1956) in Colorado, collected and tested 1,361 *C. tarsalis* females from abandoned mines, 15 December 1953 to 9 February 1954. The temperature in one mine was about 52° F., and the relative humidity fluctuated from 30 to 80 percent. They report one isolation of WEE virus from a pool of 50 of 587 mosquitoes from three mines on 30 December. Although intensive studies appear to have ceased in February, a few mosquitoes were observed in mines after the date (not stated) in spring when *C. tarsalis* was on wing.

Dow *et al.* (1956) in northern Utah, studied small numbers of *C. tarsalis* females, along with *C. pipiens* and *Anopheles freeborni,* during the winter months of 1954–55 in two abandoned mines where Fahrenheit temperatures were in the 40's and 50's. They suggest that *C. tarsalis* in these mines did not represent a successful overwintering population, presumably because of activity induced by moderate temperatures and because the numbers diminished to zero, presumably by death, toward the end of the winter. They suggest further that the species should survive best in natural shelters where it would be inactivated by cold and conclude "If *Culex tarsalis* overwinters successfully in mine tunnels, it survives in spite of excessively high mortality. It seems more likely that the normal winter habitat is still unknown."

Reeves *et al.* (1958) in Kern County, California, following a 5-year study, report isolation of WEE virus from *C. tarsalis* in

all months except December. Mosquitoes were collected from a variety of natural and artificial shelters. Sources of winter collections are not specified. Although they do not refer to the overwintering population as being in a state of "hibernation," they do note that winter temperatures and possibly other climatic factors are sufficiently rigorous to produce marked changes from normal summer behavior of the species. Male die-off in November and December is concurrent with diminished blood feeding by females which become more difficult to find through the winter. On the coldest days, at or near freezing, females in exposed places are quiescent and incapable of flight. The authors do not imply that virus found in overwintering *C. tarsalis* was due to infection acquired in late summer or fall, but on the contrary offer evidence that virus was probably acquired from infective blood meals at the onset of feeding in late January and February.

Field Observations. An excessive population of *C. tarsalis* in Grant County, Washington, during the summer of 1956 stimulated fall and winter search for hibernating individuals. Since three of the above reports (Keener; Blackmore and Winn; Dow *et al.*) record finding the species in winter only in artificial shelters, and since *C. tarsalis* presumably was here long before such winter quarters were available, an effort to discover natural hibernation sites seemed highly desirable as well as essential to a better understanding of the biology of the species.

Our activities were confined to an area of some 100 square miles in the vicinity of Moses Lake at an elevation of 1000 to 1500 feet. The land, half of which is cultivated, is semi-arid and slightly rolling. It is further characterized by abundant volcanic outcrops representing a portion of the vast lava fields of the Northwest, and by two lakes, several ponds, and two creeks. Irrigation ditches traverse the area.

The *C. tarsalis* population was under surveillance from July 1956 to April 1957. From late July until mid-September about 12,000 *C. tarsalis* females were collected and tested for virus. The WEE virus incidence was low, somewhat less than 0.3 percent.

When seasonal mosquito activity ceased, search for hibernators was begun. The *C. tarsalis* population disappeared in late September even though conditions were suitable for survival outside. During the last week of the month, temperatures ranged from 48° to 83° F. and precipitation was absent.

Searching was regular and systematic from 29 September to 7 October. Temperatures fluctuated from 36° to 78° F. and no precipitation occurred. Although emphasis was placed on natural cover, a wide variety of likely hibernation sites was investigated. Shelters inspected were animal burrows, rock crevices, standing vegetation, piles of sloughed rock at bases of outcrops, man-made rock piles, haystacks, lumber piles, chicken houses, basements, storage cellars, cisterns, outbuildings, subfloor space of houses, etc.

Mosquitoes were found only in natural and artificial rock piles, and in subfloor spaces. Volcanic outcrops with piles of loose rock at their bases (Fig. 1) varied from less than a hundred to thousands of square feet in area, and from less than 5 to more than 20 feet in height. Man-made rock piles (Fig. 2) formed by clearing fields for cultivation, ranged from 2 to 5 feet high, and the largest studied covered about 4,000 square feet. Although we were more interested in establishing presence of mosquitoes than numbers, it was immediately obvious that about 4 times as many *C. tarsalis* females were in rock piles than under floors of houses. As a rule, as soon as one mosquito was discovered another site was investigated.

During this early fall search-period, *both* sexes of *C. tarsalis, Culiseta inornata,* and *Anopheles freeborni* were found in rock piles at depths from 1 to 3 feet. The abdomens of all *C. tarsalis* females were full of fat bodies. In this same period, advanced-stage larvae of *C. tarsalis, C. ter-*

FIG. 1.—A natural hibernation site of *Culex tarsalis:* Volcanic outcrop with loose rock at base.
FIG. 2.—An artificial hibernation site of *C. tarsalis:* Man-made rock pile.

ritans, C. pipiens, and *C. inornata* were observed in various breeding places.

Observations were discontinued until 7 December when both natural and artificial rock piles were again examined. Temperatures on this day ranged from 2° to 17° F. About 3½ inches of snow had fallen 2 days before. In an artificial rock pile one female *C. tarsalis* was found at a depth of 4½ feet, and in a natural rock pile a female was discovered just beneath a surface rock. Both contained fat.

Winter inspections were made from 1 January to 5 January. Temperatures ranged from 25° to 37° F., with no precipitation. Rock piles, a basement, and storage cellars were searched. Seven of 11 artificial rock piles and each of 2 natural rock piles, yielded healthy, though quiescent, *C. tarsalis* females. These were taken at depths from 1½ to 3 feet. In one instance a mosquito was resting on the under surface of a small rock whose upper surface was covered with frost. No mosquitoes were found in a basement and 4 storage cellars.

The spring investigation for emerging hibernators was begun on 17 March. Temperatures ranged from 40° to 48° F., with a little rain. *C. tarsalis* females were found in an artificial rock pile, and a single female was taken in flight in a chicken coop. The following day (temperatures 34° to 56° F.) *C. tarsalis* females were taken from natural rock piles, but none were found in 4 chicken coops and several other outbuildings.

On 19 March an intensive search of rock piles was organized and daily collections were made through 1 April. Air temperatures ranged from 29° to 63° F., with a little rain in the last few days. During this period temperatures in a man-made rock pile, 2½ feet below the surface, ranged from 36° to 47° F. and the relative humidity fluctuated from 37 to 100 percent. A total of 555 *C. tarsalis* females were taken by aspirator. Collecting was slow and tedious and yielded only 1 to 3 mosquitoes per man hour. The largest number taken on any one day was about 100. All were examined for undigested blood and developing ova.

Early in the period mosquitoes were torpid and easily collected, but toward the end they became so active that less than half of those discovered could be captured. Until 28 March, the population in rock piles seemed to be relatively constant, as estimated by the numbers found in proportion to man-hours and effort expended. After this date, the population decreased rapidly until 1 April when only 2 mosquitoes were found in a half day.

The first blood-fed individual was found among 100 mosquitoes collected on 27 March, approximately when the rock-pile population began its rapid decline. On 29 March, 1 of 50 had blood, and 7 of 25 collected 30 and 31 March contained either blood or eggs. Also, on these last two days, 15 *C. tarsalis* collected in chicken coops had blood.

C. tarsalis and *A. freeborni* were about equally abundant in rock piles. *C. inornata,* usually present, and obviously an earlier emerger from hibernation, was not seen during the spring inspection; a lone specimen of *C. incidens* was noted in late March. In the spring, *A. freeborni* became active at the same time as *C. tarsalis.* No males of any species were found after the initial fall observation.

With almost no exception, all overwintering *C. tarsalis* females had fat bodies, no undigested blood or developing ova, and externally, had the appearance of adults recently emerged from pupation, i.e., they were unfaded, fully scaled, and had intact wings and other appendages.

During the spring study period, frequent inspections of breeding sources were made. No pre-adult stage of *C. tarsalis* was found, but early stage larvae of *Culiseta inornata, Aedes increpitus,* and *A. dorsalis* were commonly collected.

Field activities were discontinued on 2 April because of the disappearance of the hibernating population and the appearance of blood-engorged and gravid individuals.

On January 11, 3½ inches of snow fell, and from this date below-freezing tem-

peratures persisted until 5 February. An additional 5 inches of snow fell on 19 January and another 1½ inches at the end of the month. The most severe winter weather occurred the last half of January when temperatures ranged from —20° to 21° F. for 16 days. Facilities were not always available to record internal rock-pile temperatures throughout the course of our observations, and furthermore, disturbing hibernation sites more than actually necessary seemed undesirable. However, there is little doubt that at least part of the rock-pile population was occasionally exposed to below-freezing, if not below zero, temperatures.

All of the nearly 600 *C. tarsalis* collected from 29 September to 1 April were tested for WEE virus. Blood-engorged and gravid females were tested separately. The results were negative. The first 18 days of September, just before the cessation of summer activity, we collected 2145 mosquitoes of which 7 pools of 50 each had virus. Based on these figures as well as on the incidence of virus in *C. tarsalis* throughout the entire summer, at least 2 infected mosquitoes should have been in the overwintering sample tested.

DISCUSSION. A brief dictionary definition of the relative term "hibernate" is, "to pass the winter in close quarters, in a torpid or lethargic state." The mosquitoes of the rock-pile population fulfilled these requirements, and the factors indicative of true hibernation proposed by Blackmore and Winn (1956), i.e., accumulation of fat bodies, lack of feeding, absence of males, quiescence, and temperatures prohibitive for survival outside, applied equally well.

Disappearance of the summer population when temperatures ranged from middle 40's to high 80's and emergence of the hibernating population when temperatures ranged from high 20's to low 60's suggest two physiologically different populations and that air temperature is not the fundamental motivating factor for these phenomena. Bennington, Blackmore & Sooter (1958) in Colorado have experimental evidence that air temperatures alone do not stimulate emergence of hibernating *C. tarsalis* and they have reached some interesting conclusions as to the principal factor involved. Causes of the usual abrupt disappearance of the summer population and factors stimulating adults to enter hibernation require additional study.

Although inconclusive, our findings (fresh and unworn condition of hibernators; absence of undigested blood and developing ova; presence of larvae in fall; failure to isolate virus from hibernators, probably not critical because of size of sample and low virus potential) support rapidly accumulating evidence that blood-engorged *C. tarsalis* do not hibernate successfully, hence are not a mechanism for maintenance of virus through the winter.

Parallel field and experimental observations by us in western Idaho and central North Dakota, showed that fat formation in *C. tarsalis* in late summer and fall accompanies reluctance, if not refusal, to take blood. In one experiment, only 1 of 610 fall-reared females took a minute quantity of blood after several days starvation. Studies by Bellamy *et al.* (1958) suggest that blood-feeding in autumn is prejudicial to survival through the winter, and Bennington *et al.* (1958) state, "Segments of this population in cages revealed that this species [*C. tarsalis*] developed fat bodies without blood. Other observations indicate that in this species fall broods emerge, mate, take carbohydrate and the females enter a diapause to develop fat bodies. These prepared females hibernate and emerge the next spring to take their first blood meal for ovarian development."

Our discovery of a natural hibernation site of *C. tarsalis* in the Columbia Basin unfortunately does not provide a lead to hibernation sites in the vast areas devoid of outcrops and rock piles throughout the range of this mosquito. Where it spends the winter in the plains of North Dakota and the irrigated valleys of western Montana and Idaho (regions under surveillance for some years) is still a mystery.

Summary. First observations on a population of *C. tarsalis* in a state of true hibernation in a natural site (loose rock at bases of volcanic outcrops) in the Columbia Basin are reported.

Circumstantial evidence is presented that *C. tarsalis* is not *the* mechanism for maintenance of encephalitis viruses through the severe winters of northern climates.

Acknowledgment

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MOSQUITOES IN SEWAGE STABILIZATION PONDS IN THE DAKOTAS

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During recent years, the use of stabilization ponds as a method of sewage treatment has grown rapidly in popularity throughout the country. This growing popularity has been especially pronounced in the Dakotas and other states in the Missouri River Basin. Although the use of stabilization ponds is an accepted method of treating sewage in the Northern Plains area, some concern has been expressed relative to the possible public health hazard of these ponds from the standpoint of mosquito potential.

To evaluate the mosquito potential of sewage stabilization ponds, a special field survey was carried out in the Dakotas during the period July 30–August 8, 1956. Participating in this survey were representatives from the Robert A. Taft Engineering Center and the Communicable Disease Center of the U. S. Public Health Service. In addition, one of the district engineers of the South Dakota Department of Health assisted in the inspection of three installations in the southwestern portion of the state.

Of the 26 stabilization ponds studied, 14 were located in South Dakota and 12 in North Dakota. These facilities represented about 35 percent of all the waste stabilization ponds in the Dakotas. The territory inspected included each of the four geographic sections of the two states and included both the northern Great Plains and Central Lowland physiographic provinces. The ponds varied in age from less than 1 year to 8 years and in size from 1 to 135 acres.

The procedure used in sampling was to dip around the pond margins, the number of dips at each facility ranging from 25 to 200. In a few of the shallow ponds that contained weed growth throughout, the inspection was not limited to the marginal areas. Observations were made on the relative amount of vegetation in each pond. The results of the survey are summarized in table 1. The basis for classifying the vegetation was as follows: If the pond were free of both emergent aquatic plants and terrestrial vegetation, it was classified as *none* (fig. 1); if the vegetation were sparse, *light* (fig. 2); and if abundant, *heavy* (figs. 3, 4, 5, and 6). Mosquito production was classified as: *negative* if no larvae or pupae were found; as *light,* if most of the dips were negative; as *moderate,* if many were positive; and *heavy,* if virtually every dip contained larvae or pupae.

Eighteen of the ponds (69 percent) were found to be positive for *Culex tarsalis,* the common encephalitis mosquito of the western states. Of the positive ponds, mosquito production was classified as light in 8, moderate in 2, and heavy in 8. Over 99 percent of all the mosquitoes collected (735 specimens) were *C. tarsalis.* Other species collected were 2 specimens each of

TABLE 1.—Summary of entomologic inspections at sewage stabilization ponds in South Dakota and North Dakota, July 30–August 8, 1956

State	Place	Marginal Vegetation	No. of Dips	Mosquito Production
South Dakota	Wall	Heavy	150	Moderate
" "	Philip	Heavy	75	Heavy
" "	Kadoka	Light	60	Light
" "	Burke	Light	95	Negative
" "	Freeman	Heavy *	65	Heavy
" "	Beresford	Light	150	Light
" "	Sioux Falls	Heavy	85	Light
" "	Redfield	Heavy *	70	Heavy
" "	Isabel	None	25	Negative
" "	Bison	Light	35	Negative
" "	Lemmon	Light	140	Negative
" "	Veblen	Heavy	50	Moderate
" "	New Effington	Heavy *	30	Heavy
" "	Sisseton	None	30	Negative
North Dakota	Assumption Abbey	None	35	Negative
" "	Elgin	Heavy	165	Heavy
" "	Dunn Center	Light	120	Light
" "	Watford City	Heavy *	100	Heavy
" "	New Town	Heavy	100	Light
" "	Flaxton	Heavy *	70	Heavy
" "	Rolla	None	25	Negative
" "	Maddock	Heavy	200	Light
" "	Larimore	Heavy	80	Heavy
" "	Portland	Light	60	Negative
" "	Jamestown	Light	130	Light
" "	Wishek	Heavy	80	Light

* Weed growth throughout pond.

Fig. 1.—Stabilization pond with no vegetation.
Fig. 2.—Pond with light vegetation along margin.
Fig. 3.—Pond, with terrestrial plants, in the process of filling.
Fig. 4.—Emergent aquatic plants along shore of pond.
Fig. 5.—Heavy growth of terrestrial weeds along pond margin.
Fig. 6.—Heavy growth of aquatic plants throughout pond.

Aedes campestris, Aedes dorsalis, and *Culex restuans.*

In all cases where mosquito production was found, vegetation (terrestrial and/or aquatic) was present. In general, the shallowest ponds contained the most vegetation. Mosquito breeding was observed under three types of situations, *viz,* (1) when the ponds were being filled but had not reached operating depth and contained terrestrial and emergent or submerged aquatic plants (fig. 3); (2) when terrestrial plants along the shore extended or hung into the water (fig. 5); and (3) when submerged and/or emergent aquatic plants grew along the shore or elsewhere in the pond (figs. 4 and 6). Production of mosquitoes was found to be negative or of little consequence when weed growth was absent or minimal (figs. 1 and 2).

From the results of the survey, it may be concluded that most sewage stabilization ponds with vegetation have a high *C. tarsalis* production potential, but without vegetation, a low mosquito potential.

RECOMMENDATIONS FOR MOSQUITO CONTROL. To minimize or eliminate mosquito breeding in sewage stabilization ponds, it is essential that weed growth be prevented or controlled. This can be accomplished by proper design and operation of the installations.

In the basic design of the pond, it is recommended: (1) that provision be made for a minimum depth of 4 feet of water during the mosquito breeding season; (2) that provision be made for relatively steep embankment slopes—*e.g.,* 1 foot vertical to 4 feet horizontal; and (3) that access be provided so that insecticiding operations may be carried out, if necessary.

In the operation and maintenance of the facility, it is desirable to: (1) provide for rapid filling of the pond; (2) eliminate vegetative growth—both emergent aquatics and overhanging terrestrial plants—by mechanical measures or by the use of suitable herbicides; and (3) utilize larvicides as necessary. Satisfactory larvicidal formulations are: diesel oil (thin layer), 1 or 2 percent oil solution of DDT, or BHC dust (3 percent gamma isomer).

SUMMARY. A survey in 25 counties in South Dakota and North Dakota was conducted to determine whether or not sewage stabilization ponds are an important source of mosquitoes. Of 26 ponds inspected, no larvae were found in 8. *C. tarsalis* was recovered from the remaining 18, mosquito production being classified as light in 8, moderate in 2, and heavy in 8. Over 99 percent of the mosquitoes collected were *C. tarsalis*. In all cases where mosquito production was found, vegetation (terrestrial or aquatic) was present. Mosquito production was negative or of little consequence when weed growth was absent or minimal. Therefore, it was concluded that stabilization ponds with vegetation had a high *C. tarsalis* production potential, and those without vegetation, a low mosquito potential.

MOSQUITOES WANTED

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SOIL TEMPERATURE AND THE EMERGENCE OF *CULEX TARSALIS* FROM HIBERNATION

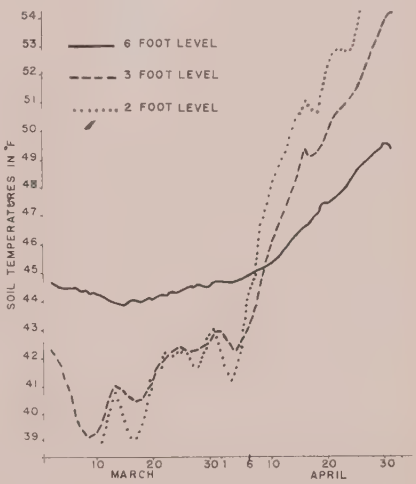
E. E. BENNINGTON,[1] J. S. BLACKMORE,[2] AND C. A. SOOTER [2]

Encephalitis investigations in the Midwest have included studies on the hibernation of *Culex tarsalis* Coquillett because this species may provide the overwintering reservoir for the western equine encephalitis and St. Louis encephalitis viruses. The fact that hibernating *tarsalis* have been collected in cellars (Keener 1952) and mines (Greeley Field Station 1955; Dow, Mail, and Richards 1956) suggests that hibernation takes place underground. Their disappearance from such places before the time of general emergence also suggests that more successful hibernation takes place in smaller sites such as animal burrows.

As these studies progressed, the emergence of female *tarsalis* from hibernation was recorded by John Blackmore who observed that each spring these mosquitoes emerged quite suddenly over wide areas of Weld County, Colorado. The data presented here show that the emergence of this species from hibernation is related to soil temperature inversion phenomena which occur simultaneously over large areas. This soil temperature inversion (see chart) is a situation which occurs twice a year, once in the spring when the surface soil becomes warmer than the subsoil and again in the fall when the surface soil becomes colder than the subsoil.

Holmquist (1928) illustrated a soil temperature inversion phenomenon in connection with ants which emerge after the inversion. McColloch and Hayes (1923) found that white grubs, which hibernate deep in the soil, emerged on March 22, 1920, and March 12, 1921. The soil temperature inversions occurred March 15–20, 1920, and March 1–7, 1921. Daily temperatures were taken at every foot to a depth of six feet and provided a timing for the control measure, plowing.

The same inversion was illustrated by Mail (1930) who indicated that many insect species hibernate above the frost line but due to a lowered freezing point could withstand the frost (Robinson 1927). McColloch, Hayes and Bryson (1928) report that in the area of their investigation the ground seldom freezes at nine inches and at twelve inches rarely gets to 32° F. making subsurface soil a good habitat for hibernation.



A SOIL TEMPERATURE INVERSION WHICH OCCURRED AT FT. COLLINS, COLORADO, APRIL 6, 1954

METHODS AND DISCUSSION. Temperature data were obtained near the Greeley Field Station with thermistors buried at six inches, three feet, and six feet. Additional soil temperature data were made available by Mr. Maxwell Parshall of the Fort Collins, Colorado, weather station. These

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temperature data when plotted show that the inversions occurred in Fort Collins March 28, 1953; April 6, 1954; April 14–15, 1955; and March 25, 1956. At Greeley, twenty miles from Fort Collins, the thermistors indicated that the inversions occurred on April 15, 1955, and March 23, 1956.

The first female hibernators were observed to emerge March 24, 1953; April 6, 1954; April 15, 1955; and March 25, 1956. These dates agree closely enough to indicate that this species does not emerge until the soil temperature has inverted and that soil temperature data could provide timing for early spring control measures.

Culex tarsalis observed hibernating in a number of mines and cellars are quite active and very few live to the date of general emergence. This is probably due to the rather higher temperatures of 50°–55° and suggests that these mosquitoes hibernate more successfully at lower temperatures of 40°–50° F. which obtain 2–3 feet below the soil surface (Dow, Mail, and Richards 1956). Khelevin (1941) observed that *Anopheles maculipennis* females died in warm basements due to premature exhaustion of the fat bodies.

In the spring of 1955 two *tarsalis* hibernators were taken by traps over wild animal burrows. In the fall of the same year a group of 223 female *tarsalis* were released in a ground level 4′ x 4′ x 4′ cage, the bottom of which was provided with three 4-inch openings to 3 vertical, 10-inch shafts extending six feet down into the soil. These females had fat bodies and disappeared from the cage in October. The next spring (1956) traps placed over the openings recovered only four mosquitoes after the temperature inversion. In this dry soil, the mosquitoes might have been more successful in shorter shafts at lower temperatures which would decrease activity and maintain a higher relative humidity.

The longevity of insects is usually lengthened by a decrease of temperature which seems also to be the main factor in the emergence of insects from hibernation (Holmquist 1928; Dowdy 1955). One explanation for the emergence of large numbers of mosquitoes shortly after temperature inversion each spring is suggested by the work of Nicholson (1934). He noted that when the temperature rose above the thermal threshold, blow flies became more fidgety until all commenced flight at the same temperature and time.

SUMMARY. *Culex tarsalis* females emerge from hibernation at or shortly following the spring soil temperature inversion. Two *tarsalis* females have been taken during the spring emergence from wild rodent burrows and four were recovered after successful hibernation in an artificial burrow.

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THE DIAPAUSE IN ADULT FEMALE *CULEX TARSALIS* COQUILLETT (DIPTERA: CULICIDAE)

E. E. BENNINGTON,[1] C. A. SOOTER,[2] AND HAROLD BAER[2]

One of the most fascinating aspects of the encephalitides is their regular seasonal appearance and disappearance. If the viruses are resident during the winter, a most logical suspect would be *Culex tarsalis,* the summer vector, acting as both vector and reservoir. In the laboratory encephalitis viruses are retained throughout life by this mosquito which hibernates as the adult female. Under laboratory conditions they live at least three months, or long enough to survive a winter and transmit virus. However, wild *Culex tarsalis* may have sufficiently different habits so that this phenomenon does not occur under actual field conditions in the Greeley, Colorado study area. The impact of natural conditions upon wild *tarsalis* is evident in the diapause or cessation of blood engorgement in fall broods. The prerequisites of *Culex tarsalis* for hibernation were investigated to determine whether this species does provide a mechanism for over-wintering viruses in nature.

It was apparent from mosquito collections made in 1954 that blood engorgement dropped rapidly in late summer, that fat bodies developed much later and that some fat bodies were developed with no blood meals. The investigation reported here was part of an ecologic study of population dynamics in relation to virus isolations and host preferences. It was carried out in 1955 by collecting weekly segments of a resting site population to find out how long these mosquitoes live, how fat bodies are developed, and to determine the extent to which re-engorgement takes place.

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[2] United States Department of Health, Education and Welfare, Public Health Service, Bureau of State Services, Communicable Disease Center, Encephalitis Investigations Unit, Greeley, Colorado.

Methods. A natural resting site was chosen for this investigation because of an apparent species preference (Brookman, 1950; Mortensen, 1953; Loomis and Green 1954). This site had been a consistently good collecting site throughout previous summers. It was the east end of an old bridge across the St. Vrain River near Platteville, Colorado. Each Monday morning the mosquitoes were collected from the end of the bridge to the first row of supports. When the population numbered in thousands, collections were made of one-half, one-quarter, or one-eighth of the area and estimates of total numbers were made.

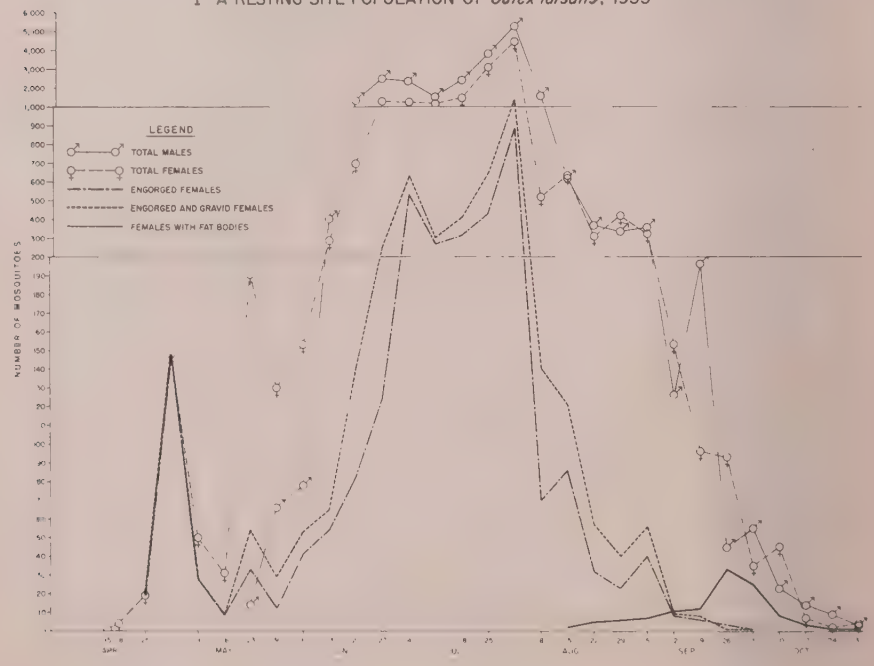
The collections were made with a large, reservoir type aspirator which caused little damage to the mosquitoes as they were carefully blown into pint-size ice cream cartons, and covered with wet towels. Upon return to the laboratory, they were lightly anesthetized with ether and carefully separated into five groups: males and blood engorged, gravid, fat, or deplete females. Numbers in each group as well as total numbers were recorded. The females presumed to be newly emerged had well defined white and dark markings which appeared in sharp contrast to the rubbed and faded appearance of older specimens. The other mosquitoes were separated as to their engorgement, gravid or fat status by the size and color of the abdomen. Fat bodies were identified by Sudan III stains.

From each weekly collection 200 females and 100 males were randomly selected, their categories recorded, and then they were allowed to revive in small cages so that any which were unable to fly could be replaced. These selections were placed in separate 18″ x 18″ x 18″ cages which were built in batteries, of wood and wire screen. These cages were constructed be-

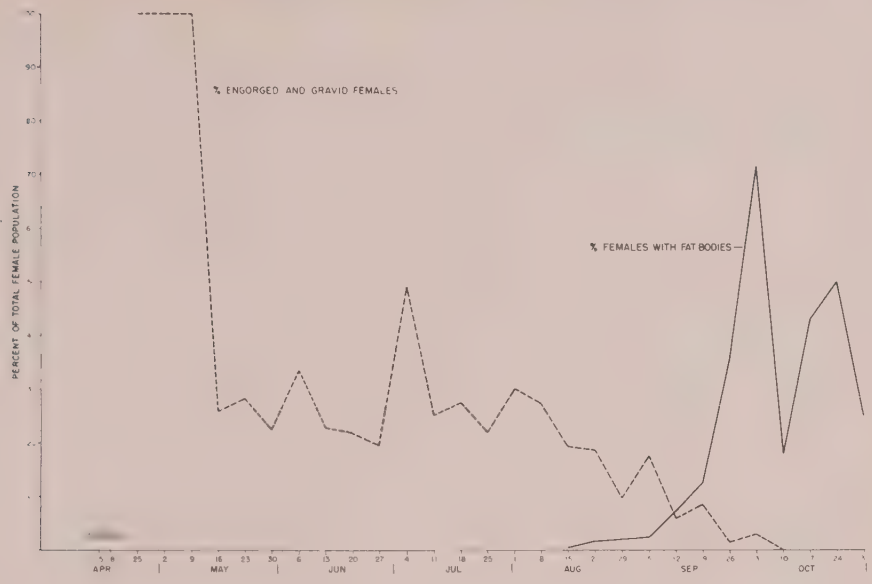
neath a small shaded bridge over permanent water which had proven to be an excellent natural resting place. Each was provided with an 8″ x 10″ pan of water and with finger bowls containing carbohydrate in the form of 10 percent sugar soaked pads. Each cage was labeled by weeks of collection from April 15 to October 10, was checked daily for egg rafts, was checked regularly for sugar solution and was provided one night each week until September 20 with an immobilized pigeon. A special row of cages was not supplied with a blood source or sugar. Three cages were used for emergence of wild larvae. The egg rafts were collected to determine the extent of re-engorgement.

OBSERVATIONS AND RESULTS. The records of the resting place collections when plotted (see graph I) showed interesting changes in the mosquito population as the season progressed. As would be expected, there was an early peak of emerging hibernators in May and another much greater peak in the female population in midsummer which dropped very rapidly in late summer. The curve indicating the numbers of engorged females in this population was almost identical with the early peak of the total female population but dropped off steadily to a peak lower than, but occurring at the same time as, the second peak of total female population. Then, about August 1 there was a sudden dramatic drop in engorgement to very low numbers, a minor peak about September 5 followed by a continued drop to almost none by October. When expressed as a percentage of the total female population, (see graph II) the engorgement rate dropped unevenly but steadily from almost 100 percent in May to less than 10 percent when fat body development began, about August 15, and continued to drop to a zero point in the first week of October. A sudden drop in the total mosquito population of males and females followed that

I A RESTING SITE POPULATION OF *Culex tarsalis*, 1955



II A RESTING SITE POPULATION OF *Culex tarsalis*, 1955



of engorgement but lagged by 30 to 40 days.

Fat body development began very slowly about August 15, proceeded to a peak about September 25, and declined by October 30.

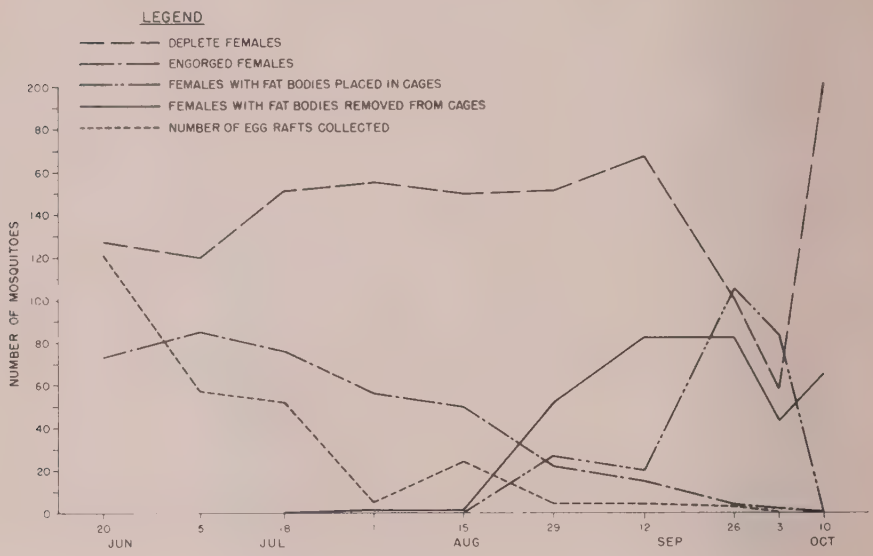
The male population began abruptly about May 22, rose quickly to remain equal to or greater than the female population except for four minor changes in September and October.

The records of the weekly population segments which were placed in cages are interesting in regard to longevity and fat body development. The recovery of egg rafts was much lower than expected and indicated that these females under the conditions imposed rarely re-engorged. Oviposition occurred at a progressively lower rate from early May to October.

The last recorded wild hibernators from the previous season were observed in the cages on May 30, June 22 and on June 30, a total of 39, 49, and 50 days in each cage. Likewise the greatest longevity recorded for females from the summer collections was 49, 66, 59, 57, 56, 53, 40, 44, 37, 70 and 71 days in each consecutive cage beginning with June; some of the later ones going into hibernation. Although some of these females lived 36–71 days at least half of them died in most cages in two weeks or less except in fall collections where longevity was increased. The greatest longevity for males was 34, 34, 31, 37, 48, 17, 23, 30 and 58 days in each cage beginning with July. The die-off time was not estimated for males but half of them were dead in less than two weeks. The high male population in late season probably indicated late emergence rather than increased longevity.

Graph III shows the composition of the weekly collections of females with respect to date, engorgement and fat body development as they were placed in each cage. The time and number of egg raft collections are indicated as well as the number of females which had developed fat bodies at the time when all of the mosquitoes were collected from each cage in October. The cages for the weekly

III DEVELOPMENT OF FAT BODIES IN CAGED *Culex tarsalis* BY WEEKS OF COLLECTION



collections were treated alike while two indicated by CHO and NO CHO, in which deplete, newly emerged females were placed, were treated differently. One cage marked NO CHO was provided only water to determine whether the fat source might be larval, the other, marked CHO was provided with 10 percent sugar in place of water in cotton pads. The females with only water died while 15 of the other group developed fat bodies with only carbohydrate.

The observations on female *tarsalis* in the cages revealed that fat body development began when the engorgement rate dropped to a very low figure and continued after no further blood engorgement could be induced in wild mosquitoes. The last blood source was offered September 20 because no blood was taken in any cage on that date. Fat body development occurred during the same time in the caged *tarsalis* as it did in mosquitoes taken from natural resting places. Prior to fat body development, many females in the cages and in resting places were observed to have engorged on some clear fluid which seemed to be a precursor of fat development. This fluid in the caged mosquitoes was the 10 percent sugar solution. Dissection revealed that fluid-filled individuals have no fat bodies or ovarian development and as they became fewer in numbers the proportion of those with fat bodies increased. All stages of development between fluid and discrete fat bodies were demonstrable.

When the mosquitoes were collected from the cages in October, more females with fat bodies were recovered from mid- and late summer groups than were originally placed in the cages while the cages with late fall collections produced somewhat fewer "fat" females than were originally introduced. Fat body development was in all cases considerably in excess of blood engorgement. Of the 100 deplete females placed in cage 20 which was never provided with a blood source, 65 were recovered with fat bodies.

DISCUSSION. Three very significant phases occurred in late season *Culex tar-*

salis populations in the Greeley, Colorado study area. They were (1) the refusal of fall broods to take blood, (2) the development of fat bodies without blood meals, and (3) the act of hibernation. The first two are prerequisite to the latter and are of most interest here. The drop in engorgement has been noted by others (Brookman 1950, Mortenson 1953) who pointed out that in California where true hibernation does not take place, there was decreased biting activity in the fall, the proportion of engorged females fell off rapidly, and many were fluid-filled. This drop in activity and blood seeking was not shared by *Culex quinquefasciatus* (Brookman 1950) or by *Culex pipiens* in the same area (Loomis and Green 1954).

Those *tarsalis* collected in late fall and winter from resting places, which would probably correspond to those truly hibernating further north, showed no evidence of blood meal or gravid condition from December through January (Mortenson 1953, Loomis and Green 1954). Brookman (1950) stated that in his winter collections the females were with few exceptions deplete and contained undeveloped ovaries. Some had fat bodies, and nearly all of them contained spermatozoa.

The observations in California on the drop in engorgement, development of fat bodies and the depletion of most winter resting females agree very well with those obtained in a more northern climate of Colorado. Some hibernation could well take place in California and not be noticed since in Colorado there is a fall dispersal at the time of fat body formation as evidenced by the collection of hundreds of females in abandoned gold mines.

The reluctance of *tarsalis* females to take blood in late summer has been at least partly responsible for early failures to colonize this species (Brookman 1950, Brennan & Harwood 1953, Lungstrom 1954) which is essentially eurygamous with a genetic stenogamic strain (Brookman 1950, Brennan & Harwood 1953). The final colony achieved by Brennan & Harwood (1953), and from which other colonies have come, seems to be stenogamic and to have other differences. Very few develop fat bodies in the fall and their ability to hibernate is questionable. They readily take blood meals in the fall and quite readily bite man in daytime. The lack of a diapause in the colony mosquitoes is mentioned by Brennan, Rush and Hubert (1954).

Wild *tarsalis* from the Greeley area, where fall broods very seldom take blood, readily develop fat bodies. They do not bite man except at dusk and hibernate successfully. It is possible that there are variants in this species as have been noted in others (Bates 1949, Richards 1941) and that differences in behavior observed in different areas may be due to selective action of extrinsic environmental factors.

The sharp drop in engorgement rate which preceded the population drop would suggest that *Culex tarsalis* is a rather unlikely overwintering host for encephalitis viruses. The sudden drop in population is a result of a very high mortality which occurs after the drop in engorgement and at a time when female mosquitoes should take the infective blood meal in order to overwinter a virus. Since individual females live at least 70 days in late season, some infected mosquitoes do without doubt live into November and December or longer in mild climates. No *tarsalis* females, however, have been found infected upon emergence from hibernation.

Some females taken in the Platteville resting place which showed only a trace of blood were dissected and found to contain a few developed ova. Since a few egg rafts were found into October, it is possible that all females taking blood oviposit (no female dissected for fat bodies had a trace of ova) and those which do not take blood—late broods—take carbohydrate and develop fat bodies to hibernate while the others probably die. Evidence for late broods is that (1) larvae were present, (2) females were predominantly unrubbed and sharply marked and (3) males which are shorted-lived usually far outnumbered the females.

From the above it seems evident that in the Greeley, Colorado area *Culex tarsalis* females from late summer and fall broods mate, take plant juice, and enter a diapause with respect to blood feeding to develop fat bodies. These females in areas of severe winters disperse to hibernate under ground, and emerge the next spring at the time of the soil temperature inversion to take their first blood meal. This habit is similar to that described for *Culiseta impatiens* and other single-brooded northern species of mosquitoes (Frohne 1953).

Frohne (1953) observed that *Culiseta impatiens, Culex territans* and *Anopheles earlei (occidentalis* of Frohne, 1953), which are many-brooded in the United States and Southern Canada, are single-brooded in Alaska and Northern Canada. In the northern climate the life cycle is characterized by refusal of newly emerging adult females to take a blood meal. They mate, take plant juice and rest (diapause) before the females enter obligatory hibernation. The biting in these species in the north is limited to emerging hibernators. It is interesting to compare *Culex tarsalis* with these species where they are many-brooded and tempting to speculate that they all impose hibernation between mating-and-fat body formation and engorgement-and-oviposition. If this is true, none of these species could be an overwintering vector or reservoir of vertebrate disease. The diapause which occurs in northern mosquitoes is entirely different from Swellengrebel's "gonotropic dissociation" in which blood is digested to form fat bodies, none being used for ovarian development (Frohne 1953).

This investigation is being continued at the Greeley Field Station to determine whether it is possible for any wild *tarsalis,* after having once taken blood and oviposited, to take carbohydrate and develop fat bodies. In two successive years large groups of engorged females have been placed in cages as previously described but have died before a fair conclusion could be drawn.

SUMMARY. A population of *Culex tarsalis* from a natural resting place near Platteville, Colorado, was studied throughout the 1955 season. Segments of this population in cages revealed that this species developed fat bodies without blood. Other observations indicate that in this species fall broods emerge, mate, take carbohydrate and the females enter a diapause to develop fat bodies. These prepared females hibernate and emerge the next spring to take their first blood meal for ovarian development. This diapause in late broods of *tarsalis* suggests that the species is a very unlikely overwintering mechanism for encephalitis viruses. The results of this study agree closely with observations recorded by other workers on the life habits of *C. tarsalis* and several northern mosquito species.

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[illegible]

[illegible]

TABLE 1—Mosquitoes collected July 5 to September 25, 1956, in New Jersey and tested for virus

Mosquito species	Total engorged *	Total non-engorged	Total mosquitoes	Total pools tested
Aedes				
canadensis		4	4	3
cantator	2	96	98	23
sollicitans	58	1390	1448	98
taeniorhynchus		5	5	5
triseriatus	1		1	1
vexans	5	175	180	50
Anopheles				
crucians	5	3043	3048	132
quadrimaculatus	7	370	377	55
punctipennis		2	2	2
Culex				
pipiens	9	389	398	64
restuans	3	130	133	46
salinarius	158	4571	4729	183
territans		71	71	37
Culiseta				
melanura	71	1807	1878	190
morsitans?		1	1	1
Mansonia				
perturbans	14	790	804	84
Orthopodomyia				
signifera		1	1	1
Psorophora				
ciliata		1	1	1
Uranotaenia				
sapphirina		381	381	26
	333	13,227	13,560	1,002

* Containing blood as evidence of recent feeding.

but recovering pony was on the premises at the time.

Taken in the same collection but negative for virus were two engorged *Culiseta melanura* and one unengorged and four engorged *Anopheles crucians*. A total of 22 other collections of mosquitoes, comprising 868 specimens in 16 species, taken resting in outbuildings or biting humans or horses on farms in Bladen, Cumberland, Brunswick, and Columbus counties, North Carolina, were all negative.

The portion of the state involved was characterized by flat pine woods and cypress swamps. Apparently without exception the premises where cases occurred and the mosquitoes were collected were within a mile or two of swampland. Of additional interest is the fact that a significant increase in mosquito breeding had occurred due to rains associated with fall hurricanes.

Laboratory studies have indicated that the *Culex pipiens-quinquefasciatus* group of mosquitoes are highly resistant or refractory to WE virus (8, 9). It is probable, therefore, that the virus isolated came from recently ingested blood rather than the tissue of any mosquito specimens in the pool. Since the collection was made in a chicken house, the blood was most likely that of chicken, but it is possible that it was from some other bird or mammal instead.

This isolation is the second of WE virus to be made from the Atlantic coast states. The first was from English sparrows in New Jersey in 1953 (10).

NEW JERSEY, 1956. A total of 13,560 mosquitoes were collected in New Jersey from July 5 to September 25, 1956, by Dr. Paul Burbutis. These were captured in 1-cubic-foot red resting boxes or New Jersey light traps in or near swampy habitats of the South River Game Farm (about 5 miles south of May's Landing, New Jersey), and from adjacent areas of similar terrain. A review of ecologic studies on EE, of which these collections were a part, is presented elsewhere (11).

These mosquitoes were shipped frozen on dry ice to the Virus Laboratory, Montgomery, Alabama, where they were identified and tested for virus in 1,002 pools. A list of the mosquitoes tested is given in table 1. Nineteen mosquito species were represented. The most abundant were *Aedes sollicitans, Anopheles crucians, Culex salinarius, Culiseta melanura,* and *Mansonia perturbans.*

Six mosquito pools yielded isolations of virus, as shown in table 2. Two of the pools containing EE virus were freshly engorged *Culex salinarius.* Since this species has been shown to be refractory to EEE infection in the laboratory (8), the virus isolated was presumed to be derived from recently ingested blood rather than the mosquito tissues.

The other four virus isolations were from *Culiseta melanura,* three of EE and one of WE. One of the EE-positive pools was composed of engorged specimens so that true mosquito infection in this instance was also questionable.

All the virus isolations were made from mosquitoes collected by light trap between July 31 and August 28, 1956, a period during which both EE and WE viruses were active in wild birds in the same area (12). The pool of engorged *C. melanura* which yielded EE virus was collected near Woodbine, New Jersey. All the other infected mosquitoes were taken on the South River Game Farm.

The recovery of EE virus from *C. melanura* further substantiates its role as an important vector. The isolation of WE virus is the second on record for this species, the first being from specimens captured in Louisiana in 1952 (3). This finding suggests the possibility that *C. melanura* may be an endemic vector of WE in the eastern part of the country.

SUMMARY. Several isolations of the viruses of eastern and western encephalitis from mosquitoes in the eastern part of the United States are reported. One isolation of WE was made in North Carolina in 1955 from a pool of engorged *Culex pipiens.* In New Jersey in 1956 two isolations of EE were made from engorged *Culex salinarius* and one from engorged *Culiseta melanura.* Pools of non-engorged

TABLE 2—Isolations of virus from mosquitoes collected by light trap in New Jersey, 1956

Place of collection	Date collected	Mosquito species	No. mosq. in pool	Feeding status	Virus	Approx. mouse LD_{50} of virus in mosq. pool	Titer of virus after mouse brain passage
South River Game Farm (SRGF)	7/31–8/3/56	*C. salinarius*	4	Engorged *	EE	$10^{2.2}$	$10^{8.8}$
SRGF	8/7–10/56	*C. salinarius*	4	Engorged	EE	$10^{1.8}$	$10^{7.5}$
SRGF	8/16–17/56	*C. melanura*	16	Non-engorged	EE	$10^{4.8}$	$10^{8.3}$
Woodbine	8/17/56	*C. melanura*	6	Engorged	EE	$10^{1.8}$	$10^{7.6}$
SRGF	8/28/56	*C. melanura*	7	Non-engorged	WE	$10^{3.1}$	$10^{6.8}$
SRGF	8/28/56	*C. melanura*	20	Non-engorged	EE	$10^{2.6}$	$10^{8.5}$

* Containing blood as evidence of recent feeding.

C. melanura also yielded two isolations of EE and one of WE.

The possible significance of these isolations is discussed briefly.

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A REVIEW OF THE MOSQUITO LARVAE OF FRANCE

I. GENERA *Culiseta, Mansonia, Orthopodomyia,* AND *Uranotaenia*

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INTRODUCTION. In recent years the study of the taxonomy and bionomics of the culicine mosquitoes of Western Europe has declined. In fact, no work which includes all of the species found in this region has been published since 1931, when Martini's "Culicidae" in Lindner's, "Die Fliegen der Palaearktischen Region," was published. Marshall's work on the species occurring in Great Britain (1938), and Natvig's book on the species of the Scandinavian countries (1948) are excellent treatises, but they deal with relatively small geographical areas and, therefore, do not include all of the species found in the central and southern portions of the Continent. Séguy (1923) reviewed the species found within the geographical limits of France, and since that time only scattered papers which deal with the mosquitoes of certain specific areas of France have appeared in the literature.

When the writer was assigned to France in 1953, for duty with the U. S. Army

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Medical Service, he was confronted with the fact that there was no publication available which would serve as a guide to the identification of all the mosquitoes found in the country. The comprehensive works of Edwards (1921 and 1932) and Martini (1931) were found to be inadequate due to synonomy, descriptions of new species, new distribution records, and various other reasons. Séguy's work of 1923 was likewise found to be of limited value for the same reasons. As the determination of the species of mosquitoes present in the entire country was of vital importance to the preventive medicine program of the Army Medical Service, the writer undertook a study of the larvae of the mosquitoes of France in the fourth larval instar.

It was not deemed necessary to consider the larvae of the genus *Anopheles* in this project as the publication of Russell, Rozeboom, and Stone (1943) was found to be completely satisfactory. These writers present a section concerning the members of the genus *Anopheles* which occur throughout the continent of Europe. Recently *A. algeriensis* and *A. hyrcanus pseudopictus* have been reported for the first time from France (Hedeen, 1957; Rioux, 1954). However, these two species are included in the paper mentioned above, and their identification should present no difficulty if encountered in France by future workers.

In order to compile a complete list of the species of mosquitoes known to be present in France, it was necessary to consult many papers which had been published in a variety of journals and periodicals. These data were combined with the collection records of the Army Medical Service made throughout the country during the period of 1953 to 1956. In some instances it was necessary to contact French workers for the loan of specimens of certain species and various publications which were difficult to obtain. In this regard, the writer is especially indebted to the following individuals: Dr. E. Noeldner, Strasbourg; Dr. E. Roman, Lyon; Dr. Jean Rioux, Montpellier. He is also grateful to Dr. E. Séguy of the French Museum of Natural History in Paris for making available the facilities of the entomology section of the Museum and for his helpful advice. Special thanks are given to Miss Thelma L. Ford of the Walter Reed Army Institute of Research, Washington, D. C., who prepared the illustrations.

The Present Study. The mosquitoes of France number approximately 53 species comprising 7 genera. The genus *Aedes,* with approximately 25 species has the largest number, followed by *Culex* with 9 species, *Anopheles* with 9, and *Culiseta* with 8. The genera *Mansonia, Orthopodomyia,* and *Uranotaenia* are represented in the French fauna by one species each.

The present paper is the first of a series of three dealing with the larvae of the mosquitoes of France. The genera *Aedes* and *Culex* will be considered in the second and third publications respectively.

Though this work is primarily concerned with the species of mosquitoes known to occur within the geographical boundaries of France, it is believed that the information given will be useful for the species occurring in other Western European countries such as Belgium, Luxembourg, Switzerland, Central and Southern Germany, and Northern Italy and Spain.

GENERIC KEY TO THE FOURTH INSTAR MOSQUITO LARVAE OF FRANCE

1. Siphon absent ..... *Inopheles*
– Siphon present 2
2. Siphon modified to pierce plant tissues; being sharp at apex and toothed on one side ..... *Mansonia*
– Apex of siphon not modified to pierce plant tissues; not sharp or toothed..... 3
3. Siphon without a pecten..... *Orthopodomyia*
– Siphon with a pecten..... 4
4. Eighth abdominal segment with a prominent sclerotized plate which bears the comb on its posterior border....*Uranotaenia*
– Eighth abdominal segment without a prominent sclerotized plate..... 5
5. Siphon with several subventral or siphonal hair tufts.....*Culex*
– Siphon with only one pair of subventral hair tufts 6
6. Siphonal hair tufts inserted at base of siphon .....*Culiseta*
– Siphonal hair tufts not inserted at base of siphon .....*Aedes*

GENUS *CULISETA* FELT

Most European workers prefer to use the name *Theobaldia* Neveu-Lemaire, for this genus. This writer, however, prefers to subscribe to the view of Freeborn and Brookman (1943) which gives *Culiseta* priority over *Theobaldia.*

This genus is represented in France by eight species, comprising three subgenera. The subgenera and the species included in each are as follows: subgenus *Culiseta: annulata* (Schrank, 1776), *subochrea* Edwards, 1921, *alaskaensis* Ludlow, 1906, *glaphyroptera* (Schiner, 1868); subgenus *Culicella: fumipennis* (Stephens, 1825), *morsitans* Theobald, 1901, *litorea* Shute, 1928; subgenus *Allotheobaldia: longiareolata* (Macquart, 1838).

KEY TO THE SPECIES OF FOURTH INSTAR LARVAE
GENUS *CULISETA*

(Morphological features are labelled in Fig. 1)

1. Distal pecten teeth long and hair-like 2
– Distal pecten teeth not long and hair-like 5
2. Lower head hair (6) more than 4-branched, the branches being more or less equal in length (Fig. 2) ..... *glaphyroptera*
– Lower head hair (6) four or less branched, one or more of the branches being distinctly longer and stouter than the others 3
3. Anal segment usually with four precratal tufts (rarely three or five) (Fig. 3) ..... *alaskaensis*
– Anal segment with two or three (rately four) precratal tufts 4
4. Distance between postclypeal hairs (4) equal to or greater than the distance between upper head hairs (5) (Fig. 1) ..... *annulata*
– Distance between postclypeal hairs (4) much less than distance between upper head hairs (5) ..... *subochrea*
5. Siphon with six to twelve stout, widely-spaced spines; anal plate not completely surrounding the anal segment (Fig 4) ..... *longiareolata*
– Siphon with a pecten not consisting entirely of stout, widely spaced spines; anal plate completely surrounding the anal segment 6
6. Basal portion of pecten obliquely inserted, not more or less parallel to ventral and dorsal edges of siphon; pecten with two to four isolated, larger teeth distally (Fig. 5) ..... *fumipennis*
– Basal portion of perten inserted more or less parallel to ventral and dorsal edges of siphon; pecten without two to four isolated, larger teeth distally 7
7. Length of subventral tuft of siphon generally less than two-fifths (⅖) the length of the siphon (Fig. 6) ..... *morsitans*
– Length of subventral tuft of siphon generally more than two-fifths (⅖) the length of the siphon ..... *litorea*

DISTRIBUTION IN FRANCE. *glaphyroptera:* This species has been reported from the vicinity of Strasbourg by Echstein (1918) and by Noeldner (1951). Séguy (1923) comments that this is a species of "Southern Europe" and should be present in the south of France. As far as can be determined, however, the two records of Eckstein and Noeldner listed above are the only two published records in existence for this mosquito from France.

alaskaensis: Noeldner (1951) records this circumpolar mosquito from the vicinity of Strasbourg, and this appears to be the only location where *alaskaensis* has been collected.

annulata: Several European workers (Edwards, 1921; Marshall, 1938; Martini, 1931; Séguy, 1923; Natvig, 1948) record *annulata* from all regions of France. Collection records of the U. S. Army Medical Service (1953 to 1956, unpublished) indicate this species to be quite prevalent in the southwestern region (Departments of Charente-Maritime, Vendee, Charente, Gironde, Vienne), and the central region (Departments of Loiret, Seine, and Seine et Oise).

subochrea: This species appears to be quite widespread throughout the country, but it was not collected during surveys of the Medical Service for the period of

1953 to 1956. Noeldner (1951) reports *subochrea* from Kolbsheim (Bas-Rhine) in Alsace, and Séguy (1923) records it from the following localities throughout the country: Troyes, Beyrouth, Perse, Ghilan, Calmors, Revigny, "Meuse." Roman (1940) has collected this species on one occasion from the vicinity of Lyon.

longiareolata: Séguy (1923) states that *longiareolata* has been recorded from the following localities: Paris, Rambouillet, Fontainebleau, Loire Inferieure, Hautes-Pyrenees, Vallee du Gave de Pau, Var, Alpes Françaises, Briançon, Menton. Callot (1944) lists it as occurring in the Department of Indre et Loire in central France, and Noeldner (1951) records it as having been found on one occasion in Alsace, near the city of Strasbourg. Roman (1940) records this mosquito from Lyon and states that it has also been reported from the Department of Calvados in the northwestern section though no specific reference to this latter record is included. Army collection records list *longiareolata* from the following localities, all of which are in the Department of Charente-Maritime on the western coast: Sablanceaux and Ars on the Ile de Re, Ile D'Oleron, Royan.

fumipennis: This widely distributed species is reported by most European workers to occur throughout Europe, except in the far north (Edwards, 1921; Marshall, 1938; Martini, 1931; Séguy, 1923). Army collection records confirm this widespread distribution, as they record the species as occurring from Cherbourg to Bayonne in the west, as well as from Pau in the south and Verdun in the northeast and Lille in the northwest.

morsitans: Culiseta morsitans is recorded by the principal European workers as being cosmopolitan in its distribution throughout Europe. The collections of the Army Medical Service confirm this, as the mosquito has been taken from widely separated regions of the country at various times of the year.

litorea: Harant and Rioux (1954) were the first to report *litorea* from France; their report was based on specimens collected from the region of the Camargue. So far as could be determined no other published records exist for the country. In June of 1955 the writer collected a large series of adult male and female specimens from beneath a bridge near Rochefort Sur-Mer (Charente-Maritime). This collection consisted of an approximately equal number of male and female specimens. Upon superficial examination, the females were tentatively identified as *morsitans,* but when males of this collection were studied in a more detailed manner, which included the examination of nine terminalia preparations, it was found that the species was *litorea. Culiseta morsitans, Culiseta fumipennis,* and *C. litorea* are so similar as adults that an error in identification, as indicated, can easily be made unless the male terminalia are studied. For this reason it appears possible that *C. litorea* may be more common in France than formerly believed.

Genus *Mansonia* Blanchard

Mansonia (*Coquillettida*) *richiardii* (Ficalbi, 1890) is the only representative of this genus known from Western Europe. The name *Taeniorhynchus richiardii* is frequently seen in the literature for this species, but most workers agree that *Mansonia* is the correct generic name. This mosquito is widespread throughout continental Europe, and it has been reported by Séguy (1923) to occur throughout France. Most of the distribution records for *M. richiardii* which are available are based on adult collections. A collection of adults was made by the writer while bathing in the sea on the western tip of the Île de Re (Charente-Maritime) in June of 1955. An intense search of the surrounding area was made in the hope of collecting the larvae. No larvae were located, although several bodies of water, which seemed to be ideal for the breeding of this species, were thoroughly searched.

The apex of the siphon of the larva of *M. richiardii* is sharply constricted and

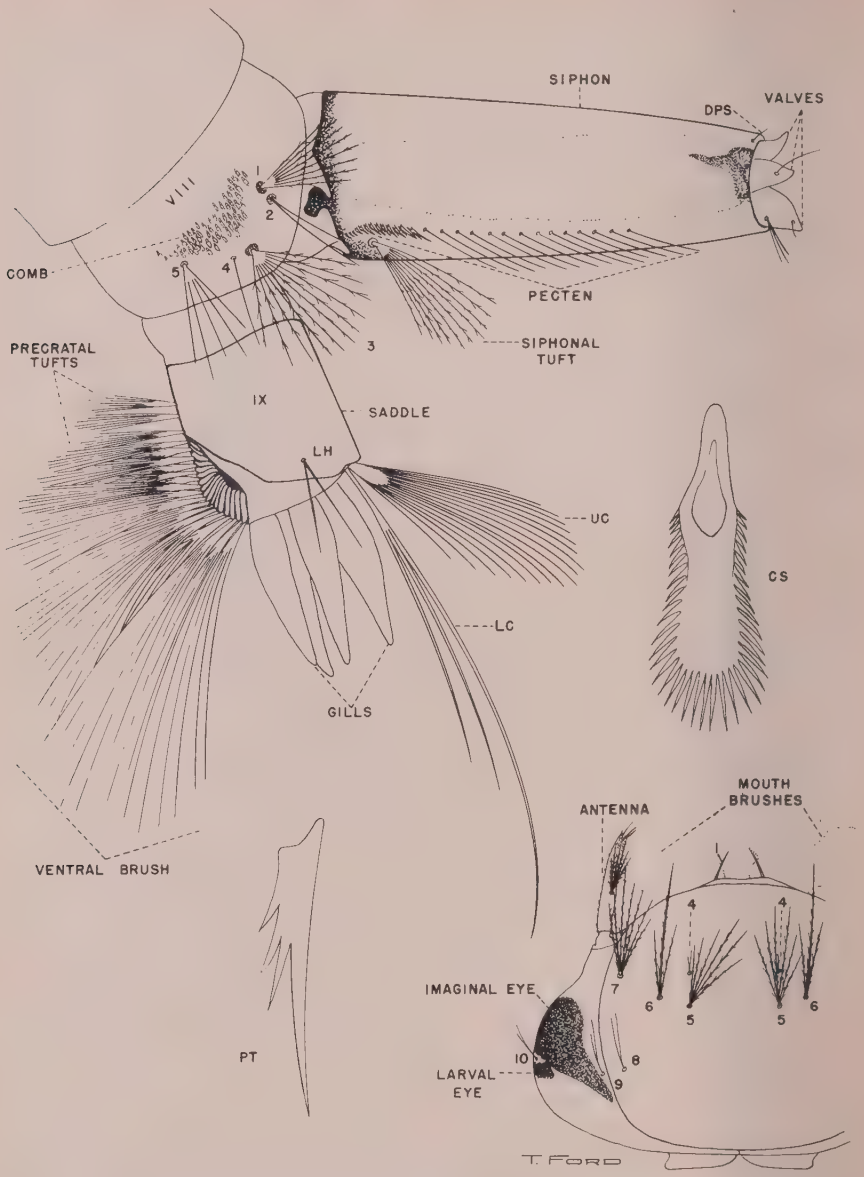

Figure 1.—Head and Terminal Segments of *Culiseta annulata* LH, lateral hair of anal segment; UC. upper caudal hair; LC, lower caudal hair; DPS, dorsal preapical spine; PT, pecten tooth; CS, comb scale.

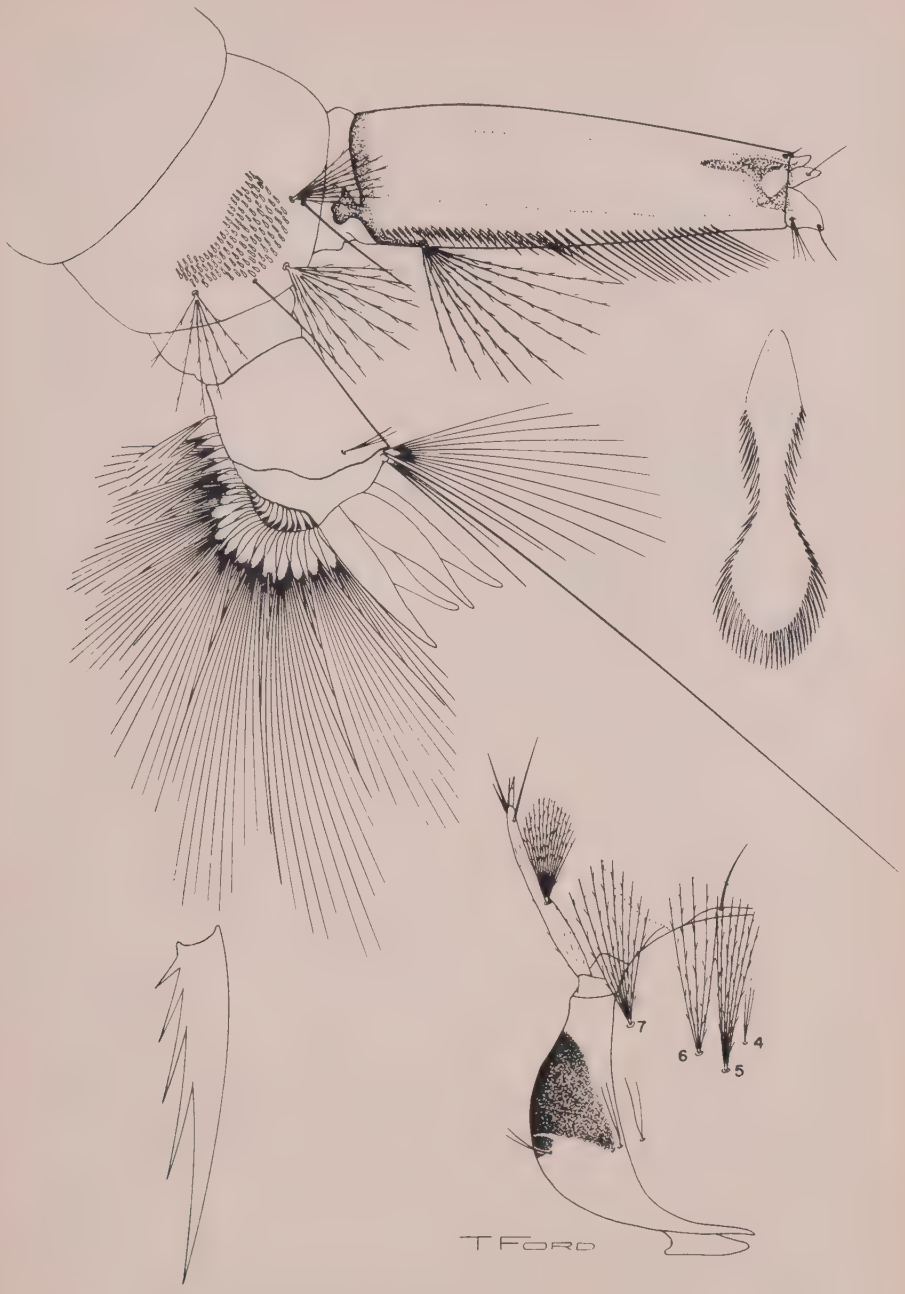

Figure 2.—Head and Terminal Segments of *Culiseta glaphyroptera.*

Figure 3.—Head and Terminal Segments of *Culiseta alaskaensis*.

Figure 4.—Head and Terminal Segments of *Culiseta longiareolata*.

Figure 5.—Head and Terminal Segments of *Culiseta fumipennis*.

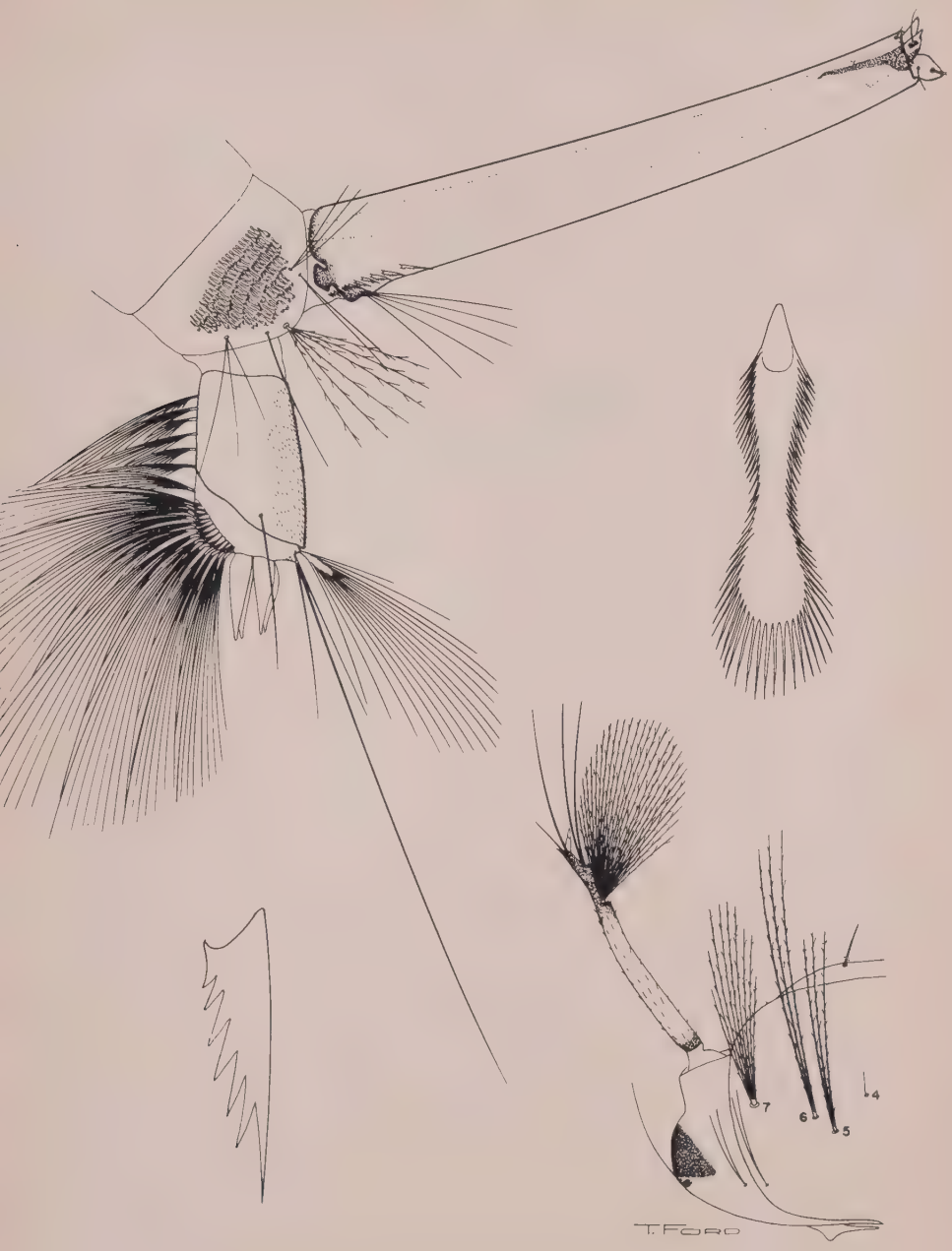

Figure 6.—Head and Terminal Segments of *Culiseta morsitans*.

Figure 7.—Terminal Segments of *Orthopodomyia pulchripalpis*.

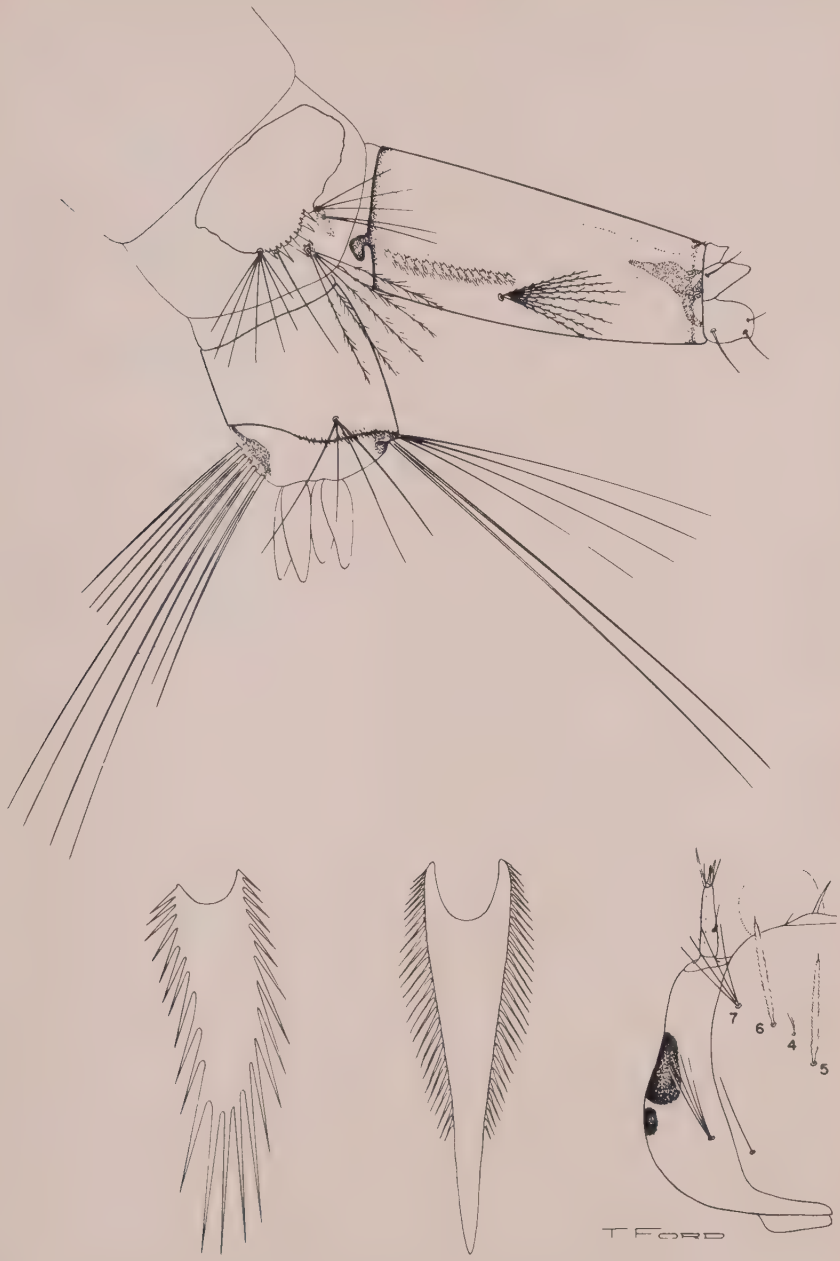

Figure 8.—Head and Terminal Segments of *Uranotaenia unguiculata.*

armed with an elaborate arrangement of hooks and spines which enable the larva to remain attached to the submerged portion of aquatic plants during the entire course of development of the immature stages. Because of this peculiar modification of the siphon it is the belief of the writer that it is impossible to confuse this mosquito with any other species occurring in Western Europe.

Genus *Orthopodomyia* Theobald

Orthopodomyia pulchripalpis Rondani, 1872, (Fig. 7) is the only species of this genus reported from Western Europe. Prior to this paper, only two records of *O. pulchripalpis* apparently existed from France. Séguy (1923) reports it from Marly in the vicinity of Paris, and Callot and Van Ty (1944–45) record it from the Department of Indre et Loire in the central portion of the country. In March of 1955 the writer made a routine collection of tree-hole breeding mosquitoes from a cavity in a large sycamore tree, (*Platanus acerifolia*) in the vicinity of the city of La Rochelle. Upon examination of the collection in the laboratory six specimens of this species were recovered. The collection also included numerous larvae of *Aedes geniculatus* and one specimen of *Anopheles plumbeus.*

The identification of this mosquito in the larval stage should present little or no difficulty when it is encountered in the region of Western Europe. It may be quickly distinguished from other mosquito larvae by its pinkish color and the absence of a pecten.

Genus *Uranotaenia* Lynch—Arribalzaga

Like the two preceding genera, this genus is represented in the French fauna by a single species, *Uranotaenia unguiculata* Edwards, 1913 (Fig. 8). This mosquito was previously regarded as an Eastern Mediterranean species. It has been recorded as far east as Iraq (Khattat, 1955). Its presence in France was first reported by Rioux (1954) from the Bas Langue in the southern region of the country. Doby (1955) has confirmed its presence in this region by making a subsequent collection. This mosquito was not collected in any of the surveys conducted by the Army Medical Service during the period of 1953 to 1956.

The larva of *U. unguiculata* is not readily distinguished from the larvae of several species of the genus *Aedes* except in the fourth instar. In the fourth instar the characteristic chitinized plate appears on the eighth abdominal segment, and this plate bears the comb on its posterior border.

SUMMARY. A review of the larvae of the culicine mosquitoes of France which belong to the genera *Culiseta, Mansonia, Orthopodomyia,* and *Uranotaenia* is presented. A key for distinguishing all the genera reported from the country is included as well as a key to the species of the genus *Culiseta.* Distinguishing characteristics of the species of the other genera included in this paper are emphasized, and a brief consideration of the genus *Anopheles* is included.

Distribution records reported by previous writers are compiled, and collection records of the Army Medical Service during the period of 1953 to 1956 are presented.

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PARTICLE SIZE STUDIES USING A NON-THERMAL AEROSOL FOG GENERATOR DESIGNED FOR CONTROL OF ADULT INSECTS

LAFE R. EDMUNDS, Principal Entomologist, ROBERT G. HAHL, Senior Mechanical Engineer and HARRY N. LOWE, Jr.
Chief

Sanitary Engineering Branch, Engineer Research and Development Laboratories, Fort Belvoir, Virginia

Introduction. Insecticidal aerosols are widely used by the Military Forces in outdoor applications for the destruction of adult populations of mosquitoes and other flying insects. From extensive use of available commercial devices it was concluded that the needs of the Military Forces could be met best by the development of a non-thermal aerosol generator providing for ready selection of the particle size making up the aerosol. When large quantities of insecticide are dispersed annually, as in the military operation, it is important that the correct particle size for maximum kill be achieved. For adult mosquitoes this is believed to be in the range of 20–30 microns mmd. This paper describes one in a continuing series of studies of equipment and methods of producing controlled sized particles in an insecticidal aerosol.

Description of Equipment. The equipment used for these studies was a non-thermal aerosol generator designed and developed by Messrs. Robert G. Hahl and Harry N. Lowe, Jr., of the U. S. Army Engineer Research and Development Laboratories, Fort Belvoir, Virginia. The unit consists of a gasoline engine, a blower, an insecticide reservoir and pump, a unique but simple flow control system assuring positive control of the liquid flow rate and a nozzle and discharge assembly that can be adjusted to any desired position (Fig. 1).

The nozzles permit the use of a low

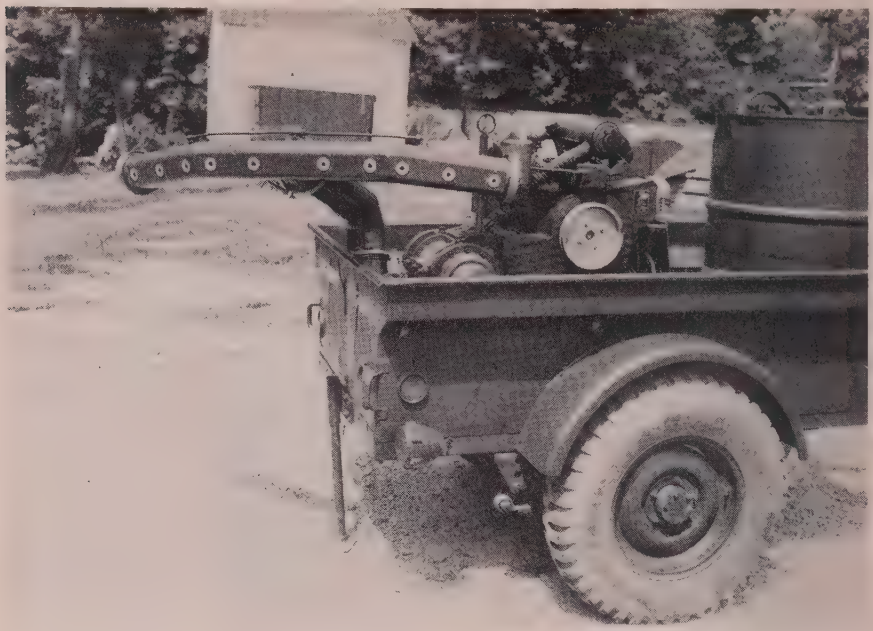

FIG. 1.—The Fogger, Insecticide-Aerosol, Gasoline-Engine-Driven, Outdoor Type, 40 GPH in ¼ ton military trailer. View of pneumatic atomizing nozzle boom. Particle Size Tests Non-Thermal Aerosol Fog Generator.

pressure, large volume air source to atomize the insecticide. One of the features of the nozzle is that it does not plug because there are no small openings or orifices.

The generator was designed to atomize diesel fuel oil No. 2 as the principal insecticide solvent since this material is the lowest cost and most readily available solvent in the field. Flow can be controlled at all values from 0 to 60 gph and air pressure adjusted to all values from 0 to 7 psi. From experience it was concluded that a practical operating range was from 30 to 60 gph at 5 to 7 psi.

PARTICLE SIZE TESTS AND RESULTS. Tests were first conducted at Fort Belvoir, Virginia and subsequently at the U. S. Department of Agriculture Experimental Station at Beltsville, Maryland. Results of these tests are summarized in Table 1.

To determine the size range of particles produced by the test equipment, a number of different solvents and formulations were atomized at different rates of flow and air pressures.

One part of twenty percent DDT oil miscible concentrate was added to two parts of diesel fuel oil No. 2 and atomized at 40 gph and 5 psi air pressure. The particles produced ranged from 22 microns mmd at 300 feet to 26 microns mmd at 50 feet. Using the same formulation but increasing the rate to 60 gph at 5 psi, the particles produced ranged in size from 28 microns mmd at 50 feet to 32 microns mmd at 25 feet.

When diesel fuel oil No. 2 was atomized at the rate of 40 gph and at 5 psi air pressure, the particles produced had a mmd of 20.2 microns. Increasing the rate of flow from 40 to 50 gph at 5 psi air pressure increased the mmd to 25.1 microns.

The control of particle size shrinkage, as the particles leave the nozzles and float through the air, was investigated. This

TABLE 1—Dri film slide data demonstrate particle size production of fogger, insecticide aerosol, 40 GPH

Ambient 75° F.

Slide No.	Formulation (volume)	Flow Rate GPH	Air-pressure Psi	Distance Feet	MMD Microns	Location
1	Diesel #2	40	5	50	20.2	Beltsville
2	Diesel #2	50	5	50	25.1	Beltsville
3	Diesel #2	60	5	50	*18.1	Beltsville
4	Airplane Spray	60	5	25	23.6	Belvoir
5	Air. Spr. ½ dies. #2	30	5	50	10.6	Beltsville
6	Air. Spr. ½ dies. #2	40	5	50	26.1	Beltsville
7	Air. Spr. ½ dies. #2	40	5	300	22.1	Beltsville
8	Air. Spr. ½ dies. #2	50	6	50	26.1	Beltsville
9	Air. Spr. ½ dies. #2	60	5	25	32.2	Belvoir
10	Air. Spr. ½ dies. #2	60	5	50	28.0	Beltsville
11	Air. Spr. ½ dies. #2	60	5	300	31.1	Beltsville
12	Dies. #2 19/1 SAE 50	40	5	50	23.2	Beltsville
13	Dies. #2 17/3 SAE 50	30	5	50	18.1	Beltsville
14	Dies. #2 17/3 SAE 50	40	5	10	30.2	Beltsville
15	Dies. #2 17/3 SAE 50	40	5	50	25.3	Beltsville
16	Dies. #2 17/3 SAE 50	40	5	50	*33.6	Beltsville
17	Dies. #2 17/3 SAE 50	60	5	25	37.7	Belvoir
18	Dies. #2 17/3 SAE 50	60	7	50	17.3	Beltsville
19	Dies. #2 16/4 SAE 50	40	5	10	24.2	Beltsville
20	Dies. #2 16/4 SAE 50	40	5	50	25.7	Beltsville
21	Dies. #2 16/4 SAE 50	40	5	300	27.3	Beltsville
22	Dies. #2 16/4 SAE 50	45	5	25	27.0	Belvoir
23	Dies. #2 16/4 SAE 50	60	5	25	27.6	Belvoir

* Possibly not representative samples.

was accomplished by adding varying amounts of SAE 50 lubricating oil to the diesel solvent. It was found that a relatively large amount of SAE 50 oil, 18 to 20 percent, is required to cause any significant retardation of evaporation of the atomized particles at distances of 300 feet from the machine. Addition of less than 10 percent SAE 50 oil produced no measurable effect on evaporation rates. Particle size shrinkage due to evaporation of the diesel No. 2 and other oil solvents of this type is not considered serious enough to warrant the addition of costly and scarce supplies of SAE 50 motor oil.

Discussion. Particle size measurement of aerosol particles is a painstaking task that should not be undertaken by other than thoroughly trained personnel. Even under favorable conditions it is desirable to have a check on results obtained by a second qualified investigator. This was accomplished effectively in these tests by dividing the laboratory measurements between the Engineer Research and Development Laboratories and the U. S. Department of Agriculture Experiment Station, Beltsville, Maryland. Good correlation of the measurements by the two laboratories was observed.

Control over the size of particles in an insecticidal aerosol is essential. Particles that are too small are deflected from flying insects by the air currents passing around the insects. Particles that are too large settle rapidly and have poor dispersion, the chances of hitting the insect being very much reduced. It is believed that the best particle size for killing adult flying mosquitoes outdoors is the range of 20 to 30 microns mmd. In this series of tests, particles in this range were produced at from 40 to 50 gph output at 5 psi air pressure. The tests indicated that other ranges of particle size can be produced by the test equipment by proper adjustment of the

flow rate and air pressure. There is only one satisfactory nozzle commercially available for this equipment. This precludes the possibility of changing nozzles to produce varying particle ranges.

SUMMARY. A non-thermal aerosol fog generator was designed in a series of tests to produce an insecticidal aerosol in the range of 20 to 30 microns mmd. Several insecticidal formulations were used. With 6.7 percent DDT in oil, particles produced ranged from 22 microns mmd at 300 feet to 26 microns mmd at 50 feet, when the equipment was operated at the rate of 40 gph and 5 psi air pressure. Particle size was varied by changing either or both the rate of liquid flow and the air pressure used for atomization. Addition of 5–10 percent SAE 50 oil to the formulation did not produce a measurable reduction in the evaporation rate of the particles produced.

ACKNOWLEDGMENTS. The authors are indebted to Dr. R. A. Fulton and staff of the U. S. Department of Agriculture, Beltsville, Maryland, who cooperated in this study.

THE MEMBERSHIP COMMITTEE

P. Bruce Brockway, Jr., Chairman of the Membership Committee, writes that his assistant chairman, Pat Nakagawa has been "doing an excellent job of signing up new members in Hawaii."

Since it is budget-making time for many Districts, Bruce included the suggestion that in states where such activity is permitted, "those in charge of the budget should look into the matter of memberships to be bought by the District itself. . . . Each District should realize that its primary source of information is the American Mosquito Control Association and that the Association stands ready to assist any District in any possible way; therefore, each District should reciprocate by maintaining membership in the Association. Furthermore, there are some Districts that owe their existence to the Association and they should reciprocate to the Association.

"Each member is morally obligated to let others know the full value of the Association and back this group by inducing others to join in our common battle on mosquitoes wherever it is deemed necessary."

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OPERATIONAL AND SCIENTIFIC NOTES

A New Concept in Aerial Spraying. During the summer of 1958, Jefferson County (Texas) Mosquito Control District tried a new procedure for applying mosquito adulticide by airplane.

The standard procedure for the District has been to fly swaths 100 to 150 feet apart. A solution of malathion in oil, calculated to give a deposit of 0.1 lb. per acre, was used. In our particular case, the concentrate contained 0.4 lb. of malathion per gallon of oil solution. The pressure and nozzles of the Piper Super Cub were adjusted to the proper rate, 1 qt. per acre. The maximum area possible in one day has been about 4,000 acres.

In an effort to increase the efficacy of our air spray operations, we increased the amount of insecticide per gallon and widened the space between swaths. Although the evaluation of this method is incomplete, we are satisfied with the progress. Currently, swaths spaced approximately 400 feet apart are being used. The nozzle arrangement and pressure are unchanged. The concentration of malathion has been increased to 1 lb. per gallon of oil. The rate of application of the insecticide, figured on a per acre basis of the entire area, is still 0.1 lb. per acre. No attempt is made to secure an even coverage of the entire area. Perhaps a better description of the operation is: we establish a series of barrier strips through which the mosquito must move to get out of the treated area. Probably the best procedure for applying the insecticide in these widely separated swaths is to lay the first swath as a barrier strip along the down wind side of the area being treated. Subsequent swaths will be laid at 400 feet intervals back towards the first swath.

The kill within the area has been equal to the kill with the blanket, or even, coverage method. The killing time is increased. Probably the killing time is reduced in the areas actually treated, but due to the time lag created by those mosquitoes caught between swaths wandering into the lethal concentration, the over-all time is greater. The reduction of population in a given area is slower by a matter of 2–3 hours than the original method.

Use of the new method of treatment, in an area where the people affected are able to watch the airplane, has resulted in considerable criticism. The population has been educated, during the past five years, to a low-flying airplane working a 100-foot swath. Perhaps as time goes on, the public reaction will change.

There are still many problems to be solved in connection with the new method of treatment. So far, it has been used only where the area lies at a right angle to the wind direction. We do not know the effect, if any, if the 400 feet barrier swaths are laid with the wind. We have arbitrarily kept our rate per acre the same as with the old method. We do not know the necessity for maintaining this high a concentration. Perhaps the concentration could be lowered with equally good results.

We have proven to our own satisfaction during the past summer that the 400-foot swath is effective and economical. We have been able to treat in excess of 8,000 acres per day without undue strain on the pilot. In fact, 8,000 acres takes less time than a 4,000 acre day did with the narrow swath procedure.

There are some pilot difficulties to overcome in the new method as most duster-sprayer pilots are accustomed to the narrow agricultural swath. The 400-foot swath is very difficult to estimate. Our pilot, who has become accustomed to the new schedule, prefers it to the old method. If any other District has any information on this subject, we will be very glad to hear from them.—George A. Thompson, Director, Jefferson County Mosquito Control.

Surfacing Time as a Simple Criterion of Normality in *Anopheles* Larvae.—In toxicological tests on *Anopheles* larvae a moment comes when it is essential to estimate rapidly and accurately the normality of the larvae. Movement of an insect is usually taken to indicate the presence of life but does not necessarily indicate normality. In order to make the criterion of normality objective and as simple as possible, a study was made on the speed with which normal feeding and fasting third and fourth stage larvae of the LTD strain of *Anopheles quadrimaculatus* Say (Diptera, Culicidae) rise to the surface after being pipetted to the bottom of a liter of distilled water.

The larvae were reared as previously described (Jones, J. C., 1957. A new standard for the rapid detection of DDT tolerance in *Anopheles quadrimaculatus* larvae and pupae. Mosq. News 17: 1–9.) Uniform and healthy looking larvae of the third and fourth stages were washed just before pipetting them individually with a large-mouth glass pipette to the bottom of a glass liter, beaker of distilled water. The time required for a larva to reattach perfectly to the air-water interface and assume its natural horizontal stance was taken (=*surfacing time*), and the larva was then quickly submerged again until it had undergone 5 consecutive submergences. If larvae did not rise to the surface in 5 minutes, they were brought to the air-water interface and then re-submerged. A series of numbered larvae were kept without food (fasting) in 20 ml. of distilled water for 24 to 96 hours before testing their ability to rise to the surface.

TABLE 1.—Mean surfacing time (seconds) of well-fed fourth stage *Anopheles quadrimaculatus*

Age in fourth stage	No. larvae	Submergence 1	2	3	4	5
		Seconds to rise to air-water interface				
1 hour	5	25.8	38.4	38.8	57.7	73.0
24 hours	5	12.3	23.4	47.8	40.3	33.6
	5	39.3	45.3	53.4	48.2	48.4
48 hours	5	57.5	26.8	41.8	45.3	56.8
	5	34.0	36.0	21.2	22.9	21.0
72 hours	5	60.6	73.2	59.0	68.2	86.6
	5	64.6	47.3	46.8	77.3	60.6

TABLE 2.—Surfacing time of fasting fourth stage *Anopheles quadrimaculatus* larvae

Age in fourth stage when fast begun	No. larvae	Submergence 1	2	3	4	5
			Seconds to rise to air-water interface			
		Fasted 24 hours				
1 hour	5	22.7	22.2	20.6	30.2	33.8
24 hours	5	8.2	38.9	29.0	36.7	18.8
48 hours	5	17.7	5.7	5.9	6.7	17.6
72 hours	2	42.5	10.2	35.8	20.2	8.4
		Fasted 48 hours				
1 hour	5	9.4	28.6	7.1	23.5	32.5
24 hours	5	5.1	39.9	8.9	5.2	22.9
48 hours	2	5.1	5.7	7.2	7.5	4.4
		Fasted 72 hours				
1 hour	5	15.2	14.9	72.7	85.7	159.2
24 hours	4	10.1	5.4	4.6	13.6	6.3
48 hours	1	8.2	5.0	6.4	6.0	4.6
		Fasted 96 hours				
1 hour	4	5.9	4.7	6.4	6.0	7.4

RESULTS.—Five well-fed third instars surfaced in 11 to 100 seconds (mean of 42.2 seconds). Seventeen third stage larvae, after fasting 24 hours, surfaced in 6 to 195 seconds, with a mean surfacing time of 32.3 seconds, there being no marked differences between the 5 different submergences which varied from 26.4 (standard error 7.4) to 39.7 (standard error 10.9) seconds. When these seventeen larvae were starved an additional 24 hours, 4 were found dead, and all but one of the remaining 13 had molted to the fourth stage. Four of the molted larvae when submerged remained submerged at each of the 5 trials for 5 minutes. The eight remaining fasting fourth instars surfaced in from 5 to 272 seconds, with a mean surfacing time of 68.7 seconds (fasted 48 hours). There was a 2- to 3-fold decrease in the speed with which they were able to surface after the first submergence. The single fasting third stage larva at 48 hours very rapidly surfaced in from 8 to 27 seconds (mean, 15 seconds).

Table 1 shows that most well-fed fourth instars surface within one minute or less (first three submergences), the 3-day old ones surfacing more slowly than the younger ones. Twenty-four to ninety-six hour fasted fourth instars rise to the surface significantly faster than well-fed specimens (Table 2).

On the basis of the data described in this note it is suggested that *Anopheles* larvae be considered normal if they are able to surface within 1 minute at either the 24 and/or 48 hour points in toxicological tests.—Jack Colvard Jones, Department of Entomology, University of Maryland, College Park, Maryland. Scientific Article No. A699, Contribution No. 2922 of the Maryland Agricultural Experiment Station, Department of Entomology.

RESULTS OF FIELD TESTS WITH NEW COMPOUNDS AS MOSQUITO LARVICIDES. (A cooperative project between the Entomology Research Branch of the U. S. Department of Agriculture and the

TABLE 1.—Effectiveness of several insecticides against fourth-instar *Aedes nigromaculis* and *Culex tarsalis* larvae in field tests. Average of 2 or 3 tests.

Insecticide	Percent mortality in 24 hours at indicated dosage per acre					
	0.4 lb.	0.3 lb.	0.2 lb.	0.1 lb.	0.05 lb.	0.025 lb.
	Aedes nigromaculis					
Chrysanthemumic acid esters						
6–bromopiperonyl	97		86			
Piperonyl	56		75			
6–chloropiperonyl	98		91			
6–chloropiperonyl plus piperonyl butoxide	—		97			
6–bromopiperonyl plus piperonyl butoxide	—		98	98	85	
2, 4–dimethylbenzyl plus piperonyl butoxide	—		99	98	82	
o–methylbenzyl plus piperonyl butoxide	—		—	86	69	
Organic phosphorus compounds						
Am. Cyanamid 12008	—		—	—	97	69
Dow ET–57 (sampled as ET-14)	—	100	80	72	—	
Trithion (Flowable 4)	—	—	—	62	46	
	Culex tarsalis					
Chrysanthemumic acid esters						
6–bromopiperonyl	82					
6–chloropiperonyl	44					

Bureau of Vector Control of the California Department of Public Health. David M. Hawbecker, field aid of the California Bureau of Vector Control, assisted with most of these experiments.) A number of new materials were tested as mosquito larvicides in California in 1957. The most promising were the chrysanthemumic acid esters. Chrysanthemumic acid is a synthetic material similar to that found in the chrysanthemum flowers, from which pyrethrum is obtained.

The chrysanthemumic acid esters have a very low toxicity to warm-blooded animals. They are only one-eighth as toxic as pyrethrum and one-third as toxic as allethrin.

The tests were made on fourth instar *Aedes nigromaculis* and third and fourth instar *Culex tarsalis* larvae in irrigated pastures. The test plots were 1/16 acre in size, and the water ranged from 2 to 10 inches in depth. The insecticides were applied as emulsions with a 1-gallon compressed air sprayer. Pretreatment and post-treatment counts were made by taking 20 dips in each plot. The results are presented in table 1.

Against *Aedes nigromaculis* some of these materials were effective at 0.4 pound per acre. Their effectiveness was increased by the addition of piperonyl butoxide, 5 parts to 1 part of the toxicant. Tests with *Culex tarsalis* indicate that they may be less effective against this species. However, heavier grass growth and floatage may have been partially responsible for the reduced mortality in these tests.

Three organic phosphorus compounds were also tested. American Cyanamid 12008 was the most effective of these materials. Mortalities of *nigromaculis* in these tests were slightly lower than those obtained by Labrecque *et al.* (LABRECQUE, G. C. GAHAN, J. B. and NOE, J. R. Toxicity of some new organic phosphorus compounds to anopheline larvae. N. J. Mosquito Extermin. Assoc. Proc. 43:172–175.), against *Anopheles crucians* and *quadrimaculatus*. C. M. Gjullin, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.* and Lawrence L. Lewallen, *Bureau of Vector Control, California Department of Public Health.*

RELATIONSHIP BETWEEN APPARENT MORTALITY AND DDT CONCENTRATION IN *Anopheles* LARVAE. —Parker made the interesting observation that 15 to 25 percent of *Aedes aegypti* larvae, showing signs of being mortally affected by DDT immediately after a one-hour exposure to 0.025 to .04 p.p.m., could completely recover within 24 hours. (A. H. Parker, 1957, The susceptibility of *Aedes aegypti* larvae of different ages to DDT and dieldrin, and the relevance of the results to the formulation of standardized susceptibility tests for mosquito larvae. Ann. Trop. Med. and Parasitol. 51(2):201–215.)

This preliminary note is concerned with the differences in apparent mortality of randomly selected, freshly fasting, unsexed third stage *Anopheles quadrimaculatus* Say (LTD strain) one hour and 24 hours after a one-hour exposure to ascending concentrations of DDT in ethanol suspensions and subsequent washing in two 1-liter rinses of distilled water. The exposures were made by placing ten larvae in 50 ml. beakers containing approximately 20–25 ml. of freshly prepared toxicant; ethanol content 0.1 percent.

As shown by the data in table 1 the apparent mortality differences betwen the 1-hour and 24-hour readings spectacularly increases from 15 to 92.5 percent as the concentration rises from .05 to .5 p.p.m. but that the difference begins to fall sharply when the concentration reaches 5 p.p.m. and apparently is nil at 10 p.p.m.

TABLE 1.—Apparent mortality differences at one and 24 hours after exposing third stage *Anopheles quadrimaculatus* to different concentrations of DDT for one hour

Concentration (p.p.m.)	Number of tests	% appearing mortally affected at 1 hour	% appearing mortally affected at 24 hours
0.05	20	17.5	2.5
0.10	20	37.0	10.0
0.5	20	95.5	3.0
1.25	4	92.5	0.0
2.5	2	100.0	70.0
5.0	6	80.0	56.7
10.0	3	76.7	76.7

The most significant point in this note and in Parker's data is that in *dilute* concentrations of DDT the larvae do *not* obtain a lethal dose at the same time they begin to exhibit well-developed symptoms of poisoning. It seems desirable to determine when larvae pick up a lethal dose in dilute concentrations and to study the mechanism by which highly susceptible larvae are able to recover from quantities of DDT sufficient to bring about "knockdown" symptoms. Dr. W. H. Hoskins (personal communication) has recently found that DDT susceptible *Culex* larvae convert toxic DDT to less toxic DDE. Presumably at certain internal doses *Anopheles* larvae can similarly detoxify DDT but at higher doses are unable to do so fast enough.—Jack Colvard Joncs, Dept. of Entomology, University of Maryland, College Park, Md. Scientific Article No. A702, Contribution No. 2926 of the Maryland Agricultural Experiment Station, Dept. of Entomology.

A HYBRID BETWEEN *Aedes aegypti* AND *A. albopictus*. In the Proc. R. ent. Soc. London (C) 23(6):29. 1958. the following paragraph appears: "Mr. P. F. Mattingly exhibited some photographs of a hybrid between *Aedes* (*Stegomyia*) *aegypti* and *Aedes* (*Stegomyia*) *albopictus*. He said that previous crosses between these species had had one of two results. Either they had failed completely or the offspring had appeared to be identical with the maternal species. The only exceptions were a single F_4 throwback to the paternal species and the one viable offspring of a backcross of the F_1 to the paternal species which again resembled the father. The photographs showed the only known example of a hybrid which was intermediate between the parent species. It was the sole emergent from 21,000 eggs obtained by crossing ♀ *albopictus* with ♂ *aegypti*. It was a male with the characteristic white mesonotal stripe of *Ae. albopictus* super-imposed on the lyre-shaped pattern of *Ae. aegypti*. The male terminalia were also intermediate but much closer to those of the maternal than those of the paternal species. He suggested that the phenomenon was one of rare chromosomal recombinations proving compatible with the maternal cytoplasm."—H.L.T.D.

FROM LETTERS TO THE EDITOR:

DR. KNIPE'S ARTICLE IN THE SEPTEMBER *Mosquito News*, just received, interested me greatly, particularly since, as you know, I was for some years engaged in development of this sort of equipment for the Army. During this time I had a chance to discuss sprayers with Dr. Knipe, *re* his use of them in India, and, of course, with Larry Hall of CDC and the WHO specs. Some of the things mentioned in the current article, particularly the idea of putting a second plastic hose around the primary one, instead of a wire coil, as a protection against sharp bending and premature breaking, seemed like an excellent and simple solution to an old worry.

The military model sprayer has a spring-loaded, ball valve for the prevention of dripping, which, I think, is superior to Dr. Knipe's improvisation. It has not been thought to require "too much pressure to be opened" and has been quite effectual. It is a part of the strainer assembly fitting into the nozzle, and is manufactured by the Spraying Systems Company of Bellwood, Illinois (Chicago). Their nozzles are almost universally used or imitated by sprayer manufacturers. We have not found stainless steel orifices to be less durable than brass and I cannot conceive that they are less durable than plastic. Chrome plated brass has worn nearly as well as stainless steel but plastic seems to me to be out of the question. Even brass wears so that a consistent and proper pattern of spray and uniform droplet size is difficult or impossible to maintain.

The military model of some sprayers is also equipped with a coupling which, since it is readily attached as well as detached, is usually called simply a "quick coupling" rather than a "Q.D." coupling. This quick coupling is made according to several patterns, basically about as shown by Dr. Knipe, one of them by Breco Division of the Perfecting Service Co., 332 Atando Ave., Charlotte 6, North Carolina, or of Baltimore, Maryland.

We, too, have found the quick coupling a definite advantage and many stations have added them to equipment which was not originally so furnished. —A. W. Morrill, Nov. 6, 1958.

IN REFERENCE TO YOUR "INFORMATION WANTED" note on page 259 of the last Mosquito News I have no direct data as to this type of septic tank mosquito production but some pretty good circumstantial evidence.

Both in the Kern district and the East Side district I have come across mosquito problems where there were large numbers of mosquitoes present and the heavy population was associated with one premise. The mosquitoes present were *C. 5-fasciatus* in Bakersfield and *C. 5-fasciatus* and *C. stigmatosoma* here. These species readily and regularly are produced in foul situations.

In these situations no opening could be found to the sewage system either outside, in or under the house. The only openings to be found were the vents. The septic tanks in all cases were well buried and no surface opening could be found.

Complete relief was experienced in about a week's time after pouring a pint of 5 lb. malathion concentrate into the toilet and flushing it several times. It has been necessary to re-treat every ten days to two weeks. I have three such places in this district now.

Since the adults are found in large numbers in the houses and the immediate outside area I would assume that they are moving freely in and out of the septic tanks either through the vents or some other openings which we did not find.

I realize that this is not the best evidence but thought it might be of interest.—Gordon Smith, Manager, East Side Abatement District, Modesto, Calif.

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EDITORIAL

The editor can think of no easy, subtle way of approaching the subject of this editorial. Perhaps the best thing to do would be to re-state James Thurber's idea that the English language is in need of the ministrations of highly trained technicians whom he would call "psychosemanticists" —therapeuticians who would "specialize in the havoc wrought by verbal artillery on the fortress of reason. Their job will be to cope with the psychic trauma caused by linguistic meaninglessness, to prevent the language from degenerating into gibberish, and to save the sanity of persons threatened by the onset of polysyllabic monstrosities." *

For some time now the word "disinsection" has been hovering around, and although it has bitten several authors, an effort has been made to keep it from alighting on our pages. If we allow "disinsection" we would have to allow "insection" and we would soon be reading that a pool was insected with mosquitoes, or perhaps more simply, just plain culicised, and our poor pets and domestic animals would be described as "fleaed" and "ticked" as well as "loused."

Even worse, take the word "arborvirus," which also attempted, more recently, to crawl between our pages. As everyone knows, an "arbor" is a framework where one ties grapes. Or, just as commonly, it refers to trees, and we have arbor days or tree-planting days all over the country. In medical terminology it has long been an accepted part of such terms as "arborization" meaning a branching, like the branches of a tree. So what is an "arborvirus"?—obviously it must be a pathogen affecting trees—perhaps a mosaic of some kind. But no—and here is where we need the psychosemanticist—we are told that it is made up of *ar* for arthropod and *bor* for borne, and that it is to be used to designate *ar*thropod-*bor*ne viruses!

It is true that on this basis we could simplify our language considerably, but to be consistent we would have to go all the way. The yellow fever virus, for instance, would be not only an arborvirus, but also a "culiborvirus," or more specifically an "aeaeborvirus."

And referring to the pathogens borne by *Anopheles* species, we could call them "anoborpaths"—at least until the proctologists got wind of it and objected, just as do the arborists and arboriculturists to the expropriation of the perfectly good word "arbor" from an entirely different universe of discourse.

We could go on, but to quote Thurber again, we have already arrived at "the onset of utter meaninglessness." We are not opposed to evolution or change in the language. For example, although we held out a long time against substituting "attractant" for "attrahent," we finally capitulated. No dictionary that we have contains the word "attractant," but on the other hand, virtually no author that we have contains the word "attrahent," or seems to know that it exists. And, after all, "attractant" seems like a perfectly reasonable word, it is not confusing, it can have but one meaning, and frequent use has removed from it the stigma of illegitimacy.

But respectable words such as "arthropod" and "borne" should be kept decently separated, and not forced into such an intimate relationship that the result is a bastard.

* From *Alarms and Diversions* by James Thurber.

ASSOCIATION NEWS

Plans for Next Annual Meeting

Salt Lake City

April 12–15, 1959

A Tentative Program for next April and request for suggestions was sent out on August 1 to about 200 AMCA members selected at random. The response has been very favorable and it appears that most members are in favor of the type program which is being planned. One of the highlights will be a half day session entitled "Operations Clinic" which will be a discussion of current operational problems by a panel of experts and the audience. A three minute limit will prevent any "speech making"! Questionnaires have been sent to all mosquito abatement agencies in the United States in order that the problems discussed will be those in which operational groups are most interested.

A "Paired Papers Session" will feature expert discussions on the pros and cons of various controversial issues, such as mosquito control vs. wildlife. A "Special Papers Bull Session" will involve informal round table discussions of papers of specialized interest somewhat on the order of the "Curbstone Conferences" which have been held by the Biology Section of the Southern Branch, A.P.H.A.

Some very interesting topics for submitted papers are already being received, and there will be some fine invitational papers. Among these will be a talk by Past-President Don Rees on mosquito problems in Indonesia (where he spent the past year) and a paper by George Pearce of CDC on chemical research in relation to mosquito control.

The period of the meeting, April 12–15, is usually one of pleasant spring weather in the Great Salt Lake Basin, and arrangements are being made for a number of interesting field trips. Another highlight of entertainment will be a noonday organ recital at the famous Mormon Tabernacle, which is right next to the Hotel Utah where the meetings are to be held.

Anyone having additional suggestions is encouraged to send them in promptly.—A. D. Hess, Chairman, Program Committee.

P. BRUCE BROCKWAY, M.D.

P. Bruce Brockway, M.D., father of our very active fellow AMCA member of the same name, died late in October at the age of 83.

Dr. Brockway was founder of the Toledo Area Sanitary District, and its Director since its beginning in 1945. He had laid the foundation for mosquito control work in the District as early as 1938. He was also founder of the Health Department in the Toledo Public Schools, where he served for 33 years as Director of Health. During World War I he was a Lieutenant Commander in the U. S. Navy Medical Corps.

He was a member of the Academy of Medicine of Toledo and Lucas County, Ohio State Medical Association, American Medical Association, American Public Health Association, American Mosquito Control Association, Chief of Staff at Flower Hospital and a board member of Flower Hospital, Toledo Hospital, St. Charles Hospital and Lucas County Hospital, where in the early years he supervised the Open Air Tubercular Preventorium. He was also a member of many fraternal, civic, and other organizations.

He is survived by his wife and two sons, Bradford Hamblen and Porter Bruce, Jr., and three grandsons, all of Toledo.

NEWS AND NOTES

AUSTIN W. MORRILL, JR.

BILL REEVES, ONE OF OUR MORE PERIPATETIC MEMBERS, RECENTLY TOOK OFF IN A DIRECTION DIFFERENT FROM HIS MORE ACCUSTOMED ONES and spent September attending the Sixth International Congress on Tropical Medicine and Malaria, at Lisbon, Portugal, where he presented a paper, and in visiting a number of places en route. Going over, he went by way of England, and visited the London School of Tropical Medicine and the Regional Virus Laboratory at Leeds, where he saw Dr. Barry Marmion, the director, with whom Bill had spent some time in 1952 in Australia, working on the Murray Valley encephalitis project. At the Lisbon Congress there were over 100 papers given in 10 days and some 600 or more members attended, including AMCA'ers Ralph Bunn and Roy Fritz of (more or less) the U.S.A. and Harold Trapido, now of Poona, India. Bill said that Harold got to Poona just in time to fall into the boiling middle of a variant of Russian Spring-Summer Encephalitis, a fever called Kyasanur Forest Disease, which is carried by *Haemaphysalis* ticks, kills monkeys like yellow fever and causes haemorrhagic fever in man.

Bill met, or re-met, over 20 persons from other countries who had visited the Bakersfield (California) Field Station, and a Russian delegation of 16 persons who participated freely in the meetings and demonstrated again that science easily surmounts language and ideological differences, when it is permitted to do so. During two half-day sessions, called by WHO, and of which Bill was elected Chairman, discussions centered on Arthropod-borne Viruses, and Bill says, "It is astounding how this field has developed with interest and research projects in almost all temperate and tropical areas of the world. There are now 79 viruses recognized in this group, of which 50 were discovered in the past *five* years."

In Port-of-Spain, Trinidad, Bill had a chance to visit the Trinidad Regional Virus Laboratory, where they made him think for a minute he was back in Kern County (California), by taking him out into an orange grove to catch birds. He said, however, "Their problems make ours look simple, with over 100 species of mosquitoes to worry about." This was also his first taste of the American Tropics and he says he almost went crazy trying to get good Kodachromes of bromeliads to illustrate *Anopheles bellator* source problems. "They have a mere 81 isolations of unidentified viruses from this one island," he adds.

His last stop was in some ways the most exciting, when he called at the Gorgas Memorial Laboratory in Panama to see Pedro Galindo. They went on a three-day visit to the jungle field station in Darien Province on the Colombian border. By Cessna 180 to El Real, then dugout canoe for seven hours up the Tuira River to its junction with the Playa. "Unfortunately, time limits and an impending revolution didn't permit going into their upper jungle camp, another day's trip each way." (!)

Panama City greeted him on his return with an outbreak of eastern equine encephalomyelitis in horses, which began to give Bill the creepy feeling that this was where he had come in, having been on the ground before when an outbreak began popping. He was in Trinidad where St. Louis virus reared its head. (We think this is good arranging on somebody's part!) Bill also visited the new Middle American Virus Research Unit in the Canal Zone, a cooperative endeavor by the National Institutes of Health and the Armed Forces, a study which is planned to be extended to several Latin American countries. Bill wrote us about all this in a plane en route from Miami, where the American Society of Tropical Medicine and Hygiene held its meetings from the 4th to the 8th of November and Bill presented a paper *there,* too.

A. W. A. BROWN IS ANOTHER TEACHER WHO DOES IT WALKING AROUND OVER THE GLOBE. He has now returned to his post as Professor of Zoology at the University of Western Ontario, in London, Ontario, much to their relief and pleasure, we imagine. From June 1956 to July of this year, "Tony" had been Biologist to the World Health Organization, headquartering at Geneva, with the task of promoting WHO's part in the coordination of information and the encouragement of research on the resistance problem. During the period of his "loan" to WHO, he had the opportunity of meeting about 300 entomologists throughout the world in their own laboratories. He encountered a considerable awareness of AMCA and of *Mosquito News* and where this was lacking he, characteristically, "tried to remedy it." WHO published this year his monograph on *Insecticide Resistance in Arthropods,* which consists of 240 pages and contains over 600 references in its bibliography. Also characteristically, "Tony" says merely that this is an indication of the magnitude of the problem, rather than an indication of the magnitude of his industriousness.

DON JOHNSON, ANOTHER GADABOUT WENT "AROUND THE WORLD IN 60 DAYS" EARLY THIS LAST SUMMER and finally had time to drop us a note about it. He stopped briefly in Japan to see Maynard Johnson and recruit him away from the Army and into ICA, (*We* call this proselytizing, Don, not "recruiting") for Laos. Then on to

Taiwan, the Philippines, Hong Kong, Viet Nam, Cambodia, Indonesia, Thailand, Laos, India, Pakistan, Geneva and home. Besides his own reunions in Indonesia, he brought news to the Vietnamese of their old friend Harry Stage, saw AMCA members too numerous to mention and found malaria control programs going forward at a rapid pace everywhere. Afterward, he visited the ICA—Pan-American Sanitary Bureau Malaria Eradication Training Center, which was started this year at Kingston, Jamaica. Don allows as how it is "fast becoming *the* place for the training of 'malaria eradicators' in English language in the Western Hemisphere."

Don also reminded Us of the President's Statement in His "State of the Union" Message to the Congress Early this Year in which he said, "Another kind of work of peace is cooperation on projects of human welfare. For example, we now have it within our power to eradicate from the face of the earth that age-old scourge of mankind: malaria. We are embarking with other nations in an all-out five-year campaign to blot out this curse forever. We invite the Soviets to join with us in this great work of humanity."

These are Fine Brave Words that We All are Proud to Hear even though we know that, as Francisco Baisas reports from Manila, "eradication does not appear *too* easy, now." He points out that the slow, hard work of wiping out foci in out-of-the-way places, in the Philippines especially among the "wild or semi-wild non-Christian tribes," is yet to be done.

Travis McNeel Gave Us a Word or Two by Telephone, when he was briefly in this country, about the similar program and problem in Mexico. There, they have been spraying mud walls in twenty towns in three areas, coastal, jungle and plateau, using a variety of muds and sometimes constructing special walls of special muds in the houses of certain villages. In these tests dieldrin has been holding up very well for 300 days or more at fairly low dosages and thus giving the eradication teams considerable encouragement in their hopes for success before resistance blocks them.

The Problem of Resistance Was Given A Reverse Curve by "Tony" Brown Recently in a short talk he gave before the sessions of the military entomologists gathered from all of the U. S. areas at home and overseas and representing also the Canadian Naval and Army entomologists. The occasion was a symposium on resistance as encountered by these military entomologists, given at a meeting of the Armed Forces Pest Control Board in Washington, D. C., a journey of Dr. Brown's we forgot to include earlier. As he pointed out, the military men (both uniformed and civilian) probably represented as inclusive a world report as one would be likely to get, and the general consensus was that resistance, while severe in some cases, is also a spotty phenomenon, highly irregular in occurrence and requiring close vigilance if we are not simply to aggravate it unnecessarily. Anyway, Dr. Brown's reverse slant on resistance was to comment on the findings of research workers in England and in Japan who have hit upon a phenomenon where certain resistance is inextricably linked in the chromosomes with a susceptibility to other, sometimes quite ordinary, chemicals. This may lead, wild as it may sound, to our being some day able to slug mosquitoes with an insecticide or a common household cleaning agent and know that one or the other is inevitably fatal to them! Well, maybe it won't be quite that simple, but it does open up new vistas, which are already being explored as you may have noted from Helen Sollers' list of References to literature in our September issue.

Which Reminds Us That Majors Ted Blakeslee and Bob Stapp, together with Lt. Laurence Johnston, all of the Army Environmental Health Laboratory, in June issued a bibliographic list giving 56 references to publications on resistance to insecticides. These were analysed to show alphabetically the genera and species of insects of medical importance in which resistance has developed, together with the insecticide to which it developed. Also listed were the countries from which resistance has been reported, giving the species concerned, the insecticide and the reference. The lists included only those instances in which resistance appears to be well substantiated by laboratory data.

George Thompson of the Jefferson County (Texas) Mosquito Control District, has sent in an Operational Note, which appears elsewhere in this issue and contains the interesting observation that by increasing the concentration of his insecticide and then expanding the width *between swaths,* he actually improved his results with airplane spraying. George, whose motto is, "Remember the mosquito will bite the hand that breeds it," had been having some trouble with invasions of adult mosquitoes from the Sabine marshes. (You've got to hand it to Texas. They even have overtones of Caesar's Commentaries in their mosquito control!)

Despite the Fact that the San Francisco Bay Area and Central California Had Six Months of Higher-Than-Normal Temperatures Both Les Brumbaugh and Chet Robinson reported that mosquito populations continued well under control and the major popular outcries were concerned with household breeders. Both are carrying ahead ambitious programs of source reduction and report good results in achieving public cooperation in these projects. John Brawley, who had had the disquieting experience of en-

countering parathion resistance in some fields earlier in the summer, reported that the resistance remained spotty and did not spread laterally from the original fields. Eventually, he was able to bat 'em down with a mixture of methyl and ethyl parathions and *hopes* his luck will last into next season. (Maybe we'll be spraying 'em with vinegar by then, John,—or Cobalt.) Elsewhere, other Districts reported that malathion resistance which had previously appeared seemed to be halted when the dosage was raised and so maybe there are a few bright rays in the dark clouds, after all.

ROLLIE DORER REPORTS VIA THE VIRGINIA MOSQUITO CONTROL ASSOCIATION'S *Skeeter,* that the outdoor drama entitled, *The Confederacy,* which was presented in an outdoor bowl at Virginia Beach this summer, was a testament to successful mosquito control. The newspaper report of the gala opening even commented that, "It was a beautiful night with a cool breeze, bright moonlight and no mosquitoes." As with the similar outdoor drama at Williamsburg, source reduction and hard legwork by the abatement districts, coupled with judicious spot-fogging, have made ALL THE DIFFERENCE. Rollie also reports that the VMCA's film on mosquito control is approaching its final stage, whew. And he includes the snappy note that, "The New York Times states, 'Mosquitoes prefer to bite blonds dressed in black rather than brunettes dressed in white.' It might be inferred from this statement that a bereaved blond is more luscious than a happy brunette; but perhaps *you* might like to bite a blond sometime, just to check. . . ."

DICK PETERS REMINDS US THAT THE CMCA MEETINGS FOR 1959 WILL BE HELD AT MONTEREY, CALIFORNIA, and will be open to all. The dates are the 2nd, 3rd and 4th of February and the place is a beautiful and spankingly new motel entitled, *The Mark Thomas Inn.* This is located right across the main highway from the old Del Monte Hotel, now the Navy Postgraduate School, for the benefit of out-of-staters who, we hope, will come this way.

RALPH BARR IS ANOTHER WHO ATTENDED THE MIAMI MEETINGS of the American Society of Tropical Medicine and Hygiene. Afterward he was scheduled to swing by the Vero Beach laboratory and then up to the Communicable Disease Center laboratory at Savannah, Georgia. Miiiighty nice.

DON GRANT AND TOM LAURET ARE SWINGING INTO HIGH GEAR IN RESEARCHES ON *LEPTOCONOPS,* the wee devil of a biting gnat which inhabits California's adobe soils and comes up only to make things unbearable for humans. DON and TOM have been joined by Harold E. Munsterman, another biologist, now working on his Master's at Stanford.

EDMUND STANSFIELD HAS MADE A PRELIMINARY SUMMARY OF HIS FINDINGS IN THE GREATER WINNIPEG MOSQUITO ABATEMENT DISTRICT and his light traps showed him a satisfying degree of control but also a sobering potential for invasion by mosquitoes from outside. Fortunately resistance has as yet presented him with no problem and pretreatments were found to be quite effective, as evidenced by numerous newly-hatched larvae found dead in the water after the early rains. Interesting results were also obtained with tests of detergents, which killed larvae in concentrations as low as 25 ppm. Shall we *wash* our troubles away, do you think?

ALLAN RALPH BARR, who, we told you recently, is now with the California State Bureau of Vector Control, leads our Who's Who list this time, and we learn from *American Men of Science* that he was born in Ft. Worth, Texas, in 1926. This teaches us that you can never tell when you will be running into a Texan and we had better stop making with all these cracks. He received his BS from Southern Methodist and his ScD from Johns Hopkins. Just before that he had given the Navy two years just before the close of the war (W.W. II, that is) and since he didn't elucidate, we can't either. He was instructor in entomology and parasitology at the University of Minnesota for three years, where he began to make a name for himself, and went on to the University of Kansas in 1955 where he furthered that undertaking. We mentioned elsewhere that he is now, as we write, jaunting about Florida and in no position to deny anything we might choose to write but we'll stick to the facts, ma'am. The BVC is mighty happy to have him now with them. He is a member of the Entomological Society of America, the Society of Systematic Zoologists, Society of Tropical Medicine and Hygiene, and has been an AMCA'er for five years now.

GEORGE BURTON WAS BORN IN NEW YORK CITY in 1919 and received his BS and MS from CCNY, moving over to New York University for his Ph.D. in 1946. In between he had been a high school biology teacher and a draftsman for the Navy Department Hydrographic Office, as well as seeing active Navy duty as an entomologist with malaria control units. Returning from that for the aforementioned Ph.D. and some time with the U. S. Department of Agriculture and the Pennsylvania State Teacher's College, he then shifted to the Army and saw some more active duty in Germany and Italy as Commanding Officer of the 448th Preventive Medicine Survey Detachment. He settled down momentarily after that with the Engineer Research and Development Laboratories but the old wanderlust had bitten deep into him

by now and he started on those journeys for ICA from which he has sent us so many interesting Notes from All Over. He is now (we think) in India, but we'll probably get a letter telling us differently as soon as the galley proof has been run.

Earl Mortenson was born in Pueblo, Colorado in 1923 and attended Colorado State University. After receiving his degree he came to California to the Graduate School of the University of California at Berkeley and in 1949 went to work with the Bureau of Vector Control. He was with the late, well-remembered Deed C. Thurman, Jr., in the original work on the biology of *Aedes nigromaculis* studies on irrigated pasture breeding, when this major enemy first exploded into our scene, and he remembers that period as the year of the 20-hour days. Their Turlock laboratory was home-made tables and scrounged dishpans, then. (We forgot to say that Earl saw his active Army duty with the 1st Infantry Division (Field Artillery) in the European Theatre of Operations in France, and no doubt his Army experience made this process of working with nothing but what you can rassel up for yourself a familiar one for him.) Things are different now, and Earl's headquarters is the BVC's mighty fine lab at Fresno. He is married, and has two sons, eight and five, so his other hobbies of fishing and tennis will have to wait a bit while he grows them up a little.

Lester W. Smith Is A Charter Member of AMCA and One Whom We'd All Know because of his activity in the organization even if he weren't the guy to'm we all send that little old annual contribution. He is also the Executive Secretary of the Middlesex County Mosquito Extermination Commission and has been for some 37 years. In order to achieve this, Lester thoughtfully got himself born in 1901 in Brooklyn, N. Y. It was only a step across Manhattan to New Jersey and he took this fatal step in time to attend high school in Metuchen and then go on to Mt. Hermon Prep School and Pace College. He was married in 1928 and has two children, one of whom made Lester a granddaddy about four years ago. His son, after finishing at Rollins, has gone into the Navy. It is, of course, no secret to any of us that the work in New Jersey pioneered much of the territory into which most of the rest of us plunged at various times thereafter and Lester has therefore varied his homework with special assignments with the U. S. Department of Agriculture and assisting in organizing the mosquito control work in a number of states. He is a past president of his Rotary Club and a Past Master of his Masonic Lodge, as well as past-President of the Metuchen Golf and Country Club. That last makes it pretty plain, we guess, that his mosquito control has satisfied the customers.

One of Our Newest Members Is Cornelio M. Urbino Who Is, However, No Newcomer to Mosquito Control. He was born in 1899 of poor parents in the village of Pozorrubio, Pangasinan Province, Philippines, and lost his father, who was a "Katipunero" or fighter for independence against the Spaniards and later against the invading Americans. Cornelio worked his way up through school, however, finishing at the University of the Philippines College of Agriculture, at Los Banos, south of Manila. He joined the Malaria Control section of the Department of Health in 1927, as Assistant Entomologist, and began studies of mosquito bionomics, which were continued until 1938. In 1938–1940 he was on the island of Mindoro, which is an incredibly fertile, mountainous land lying almost in the doorway of Manila Bay, but virtually uninhabitable because of malaria until recent control campaigns have unlocked the portals. His work on Mindoro incriminated *Anopheles mangyanus* as a vector, which has been confirmed by later WHO workers; this species, like *A. minimus flavirostris*, the principal Philippine vector, breeds in flowing streams, though of a somewhat different type. During the Japanese Occupation, Mr. Urbino worked on malaria control on Bataan and after the war was with the American 8th Army malaria control units, principally the 423rd Medical Composite. In 1945, about the time that the Philippines became an independent nation, he rejoined the Malaria Division of the Department of Health which was being resurrected under the capable direction of Dr. Antonio Ejercito, another member of AMCA and known to many both from their visits to him in Manila and his visits to us, here. In 1946, 1947, and 1948, AMCA'ers Ejercito, Urbino and Baisas aided and sustained the American army malaria control efforts in many ways, not the least of which was by arising at dawn to apply sprays and dusts from low flying planes and fogs from many varieties of home-made and commercially-made fog generators, with suitable population counts before and after, in an effort to find a method of penetrating the heavy growths of wild grasses and rice paddies gone wild, which concealed and protected the breeding places of the several local anophelines. (Found it, too! *Mosquito News* 12(2):110).

REVIEWS AND ABSTRACTS

HELEN LOUISE DURKEE

INSECTICIDE RESISTANCE IN ARTHROPODS. By A. W. A. Brown. World Health Organization Monograph Series No. 38. pp. 240, Fig. 29. Geneva 1958. U. S. $5.00— £1/5s. The fantastically active chlorinated hydrocarbon insecticides present us with the serious "dilemma of insecticide-resistance": the more of these insecticides that are used, the greater the amount of insecticide-resistance that will be encountered.

The long-term solution of this dilemma can come only from research—greatly intensified research—that will provide means for overcoming the hard, cold facts of genetic physiological resistance of insects to insecticides. Meanwhile, let us be thankful that all insects are not like the house fly—completely resistant to all known chlorinated hydrocarbon insecticides.

The attention that is being given to resistance to insecticides, instead of to susceptibility to insecticides, is like looking at the hole instead of the doughnut. Susceptibility to insecticides is the phenomenon by which insects, and the diseases they transmit, are controlled or eradicated. Resistance and susceptibility need to be kept in proper perspective. An unfortunate tendency has grown up, under conditions that are readily understandable, of black-listing as resistant a whole species when only a part of the population of that species has been found to be resistant. In at least one insect, *Aëdes aegypti,* its degree of resistance in different populations ranges from complete susceptibility in a huge area, to marked resistance in a much smaller, but still large area.

Even in a review in *Mosquito News* it may not be amiss to mention the fact that physiological resistance to insecticides is a genetic phenomenon that is merely revealed by insecticides, never produced by it. In most cases the vector control operator must concentrate the resistant genes very greatly before they become detectible by one or another of the quantitative procedures that have been developed to measure the degree of insecticide resistance.

There is much talk about "establishing a base line of susceptibility" of mosquitoes, especially *Anopheles,* to the insecticide to be used in any given control operation or eradication campaign. In practice, however, this never gets done because the present test methods are much too cumbersome for really widespread use, and they are nowhere near sensitive enough to detect resistant genes when they are present in the proportion of only a few parts per million.

The monograph contains a list of 692 references, published during the decade ending in mid-1957, and has four chapters: 1. The nature and characterization of resistance; 2. Taxonomic groups characteristically vectors to man (total 37 of which 23 are *Anopheles*); 3. The housefly; and 4. Taxonomic groups characteristically not vectors to man (20 groups).

The chapters are conveniently arranged, and they contain an excellent discussion of resistance in all its facets, genetic, physiological, and biochemical. In sum, the book is an excellent comprehensive summary and review of the status of insecticide-resistance in early 1958.

Surprising are the difficulties that exist in defining insecticide-resistance. The dividing line between "vigor tolerance" and incipient resistance is still a matter of guesswork. Your reviewer is surprised to learn that there is no recommended procedure for estimating the standard error of an LC_{50} that a mere earth-bound entomologist or vector control operator might use in order to determine the limits of confidence of the test results that he obtained.

Regrettable are the errors in Fig. 7 (p. 64) regarding the progress of the *Aëdes aegypti* eradication campaign in the Americas. In the text three lines are used to mention the eradication of *aegypti* from 15 countries in South and Central American, while the succeeding three pages are devoted to the DDT-resistant populations of *aegypti* that are present in the Caribbean area. There is the implication that insecticide-resistance has been a factor in the continental United States of America, whereas the truth is that eradication operations have barely been started and the only data your reviewer knows of indicate that *aegypti* is normally susceptible there. On page 14 there is an obvious non sequitur: "The outbreak of yellow fever in Trinidad in 1954 was contemporaneous with a failure to *Aëdes aegypti* on the island due to its DDT-resistance." The yellow fever in Trinidad was primarily jungle yellow fever, with the virus repeatedly isolated from wild-caught *Haemagogus sp.* mosquitoes.

Insecticide-resistance can be a serious problem —it already is in many places—and the geneticists insist that it is certain to become more serious. Be that as it may, the better our knowledge of the facts of the matter the better will be our handling of the resistance problems that confront us, until such time as more research, and better research, may provide precisely the tools that are needed to solve the dilemma.—J. Austin Kerr, M.D., Chevy Chase, Maryland.

INVESTIGATION OF EASTERN EQUINE ENCEPHALOMYELITIS. I. GENERAL ASPECTS. By Jungherr, E. L. and Wallis, R. C. Amer. J. Hyg. 67(1):1–3. 1958. In general, these studies indicate that the pheasant is a most susceptible vertebrate host and serves as a sentinel of dangerous concentrations of EEE virus for man and horses; that owing to

high susceptibility to and mortality from EEE, the pheasant is usually in the role of a terminal host with minor contribution to the contagious cycle of the disease; that the build-up of virus concentration resulting from pheasant outbreaks can be minimized by prompt anticannibalistic measures and good managerial factors to take care of their species-peculiar space and protein requirements, and that these factors should be taken into account in the elucidation of the ecology of EEE in nature.—Excerpt from text.

II. OUTBREAKS IN CONNECTICUT PHEASANTS. Amer. J. Hyg. 67(1):4–9. 1958.

III. PATHOLOGY IN PHEASANTS AND INCIDENTAL OBSERVATIONS IN FERAL BIRDS. Amer. J. Hyg. 67(1):10–20. 1958.

IV. SUSCEPTIBILITY AND TRANSMISSION STUDIES WITH VIRUS OF PHEASANT ORIGIN. Amer. J. Hyg. 67(1):21–34. 1958.

V. ENTOMOLOGIC AND ECOLOGIC FIELD STUDIES. By Wallis, R. C., Jungherr, E. L., Luginbuhl, R. E., Helmboldt, C. F., Satriano, S. F., Williamson, L. A., and Lamson, A. L. Amer. J. Hyg. 67(1):35–45. 1958. Results of a 3-year study of mosquito populations and ecology of eastern equine encephalomyelitis areas in Connecticut are summarized. Twenty farms where the disease occurred were investigated during the late summer season while virus activity was present. Year-round studies were conducted from 1953 to 1956 in representative fresh-water and salt-water swamp areas.

Each site where the disease has occurred in Connecticut has been in, or adjacent to, fresh-water swampy woodland or fresh-water swamp-forest border locations adequate for abundant mosquito breeding. The forest and woodland harbored a wide variety of migratory and resident wild bird life. The mosquito population was predominantly composed of woodland species among which *Culex restuans* and *Aedes triseriatus* were prominent. The distribution of *Aedes vexans* extended to all known infected areas, but salt-marsh species were restricted to 4 of 14 such areas investigated. The statewide distribution of *Culiseta melanura* was not established, but early summer population build-ups and feeding activity of *Culiseta* species centered around pheasant pens were observed.

Transmission in pheasant flocks during the cool fall weather beyond the peak of the mosquito season was by secondary contact transmission within the pens. However, study of mortality records of the outbreaks indicated that initial deaths occurred earlier while mosquito activity was high.

Mosquito pools totalling 5,864 specimens were prepared for virus study with negative results. It was suggested that Connecticut's forests and woodlands, which cover ⅔ of the state provide a "sylvan" ecology in which disseminating wild bird hosts and potential mosquito vectors are so widely dispersed that eastern equine encephalomyelitis remains essentially an agricultural and wildlife problem.—Authors' summary.

(A list of 62 references completes this symposium.)

NOTES ON THE BREEDING HABITS OF SOME CULICINE MOSQUITOES (DIPTERA: CULICIDAE) IN SOUTHERN GHANA. By Surtees, G. Proc. R. ent. Soc. Lond. (A) 33(4–6):88–92. 1958. 11 refs. It would seem from this survey that the mosquito species had well defined areas of breeding and within these areas preferences for particular breeding site types. For instance, *E. chrysogaster* bred most abundantly in the cocoa plantation and showed a preference for decaying cocoa husks, *E. oedipodius* also in the cocoa was almost entirely restricted to rolled leaves. *U. ornata* was to be found most abundantly alongside the reservoir, breeding in pineapple leaf axils, whilst *A. simpsoni* was largely restricted to the residential areas and bred most abundantly in banana leaf axils. *U. fusca* was only found in rock pools alongside the reservoir. Furthermore, each of the major areas seemed to have a dominant culicine species, *E. chrysogaster, U. ornata* and *A. simpsoni* playing these roles in the cocoa plantation, reservoir and residential areas respectively. It is interesting to note that within the cocoa plantation 14 culicine species were recorded, whilst only 8 different species were found in both the reservoir and residential areas.—Author's conclusions. (The 19 culicine species taken were: *Eretmapodites chrysogaster* Grah. *E. penicillatus* Edw., *E. grahami* Edw, *E. oedipodius* Grah., *E. sylvestris* Ing. and de Meil., *E. quinquevittatus* Theob., *Culex (Culiciomyia) nebulosus* Theob., *C. (Culex) ingrami* Edw., *C. (Culex) annulioris* Theob., *C. (Culex) perfidiosus* Theob., *C. (Neoculex) rima* Theob., *Aedes (Stegomyia) simpsoni* Theob., *A. (Stegomyia) apicoargenteus* Theob., *A. (Stegomyia) aegypti* Linn., *Harpagomyia taeniarostris* Theob., *Uranotaenia ornata* Theob., *U. fusca* Theob., *U. anpulata* Theob., and *U. candidipes* Edw.)

NOTES ON THE BIOLOGY OF A NEW SUBSPECIES OF *Anopheles wellcomei* (DIPTERA: CULICIDAE) FROM EAST AFRICA, AND ON THE DISTRIBUTION OF RELATED FORMS. By Gillies, M. T. Proc. R. ent. Soc. London (A) 33(1–3):9–14. 1958. 11 refs. 9 figs. A new subspecies of *Anopheles wellcomei*, geographically isolated from other forms of the species, is described from Tanganyika and Kenya. The distribution of this and of other forms is discussed, and notes are given on its behavior.

A. wellcomei crepens (subsp. n.) is most abundant during the months of May to August, following the main rainy season. The larvae have been found in permanent and semi-permanent swamps and streams in the South Pare District of Tanganyika. They show the group habit of climbing up the stems of emergent plants or stakes, thus maintaining themselves a little above the main water surface. They presumably achieve this position by means of the belts of thoracic and abdominal spicules, characteristic of members of the *distinctua-wellcomei* group. It seems that they can main-

tain themselves in position despite some degree of disturbance of the water surface.

The adult mosquitoes have been caught biting man at night on numerous occasions. Biting has been observed more frequently outside than inside houses, although it cannot be claimed that a critical comparison has been made of the two activities. They can, however, be caught most abundantly either biting cattle in the open or resting round thorn animal enclosures at night, and it seems that they are primarily zoophilic. No glands infected with sporozoites were found amongst 127 mosquitoes dissected in South Pare District. It is not impossible, however, that an occasional specimen may live to transmit malaria, as has been shown for *A. wellcomei wellcomei* in the northern Cameroons (Hamon *et al.* 1956). Moreover, Dr. A. Smith has observed *erepens* biting man in large numbers near the Ruvu River in Northern Tanganyika, even after the houses had been sprayed with residual insecticides. In the presence of other more potent vectors it could obviously be of no importance as a malaria carrier, but in localities where it is abundant it should be regarded with suspicion as potentially capable of maintaining lowgrade transmission out-of-doors, especially if it should be found that house spraying has failed to eliminate malaria completely.—Author's summary and excerpts from text.

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REFERENCES TO LITERATURE OF INTEREST TO MOSQUITO WORKERS AND MALARIOLOGISTS

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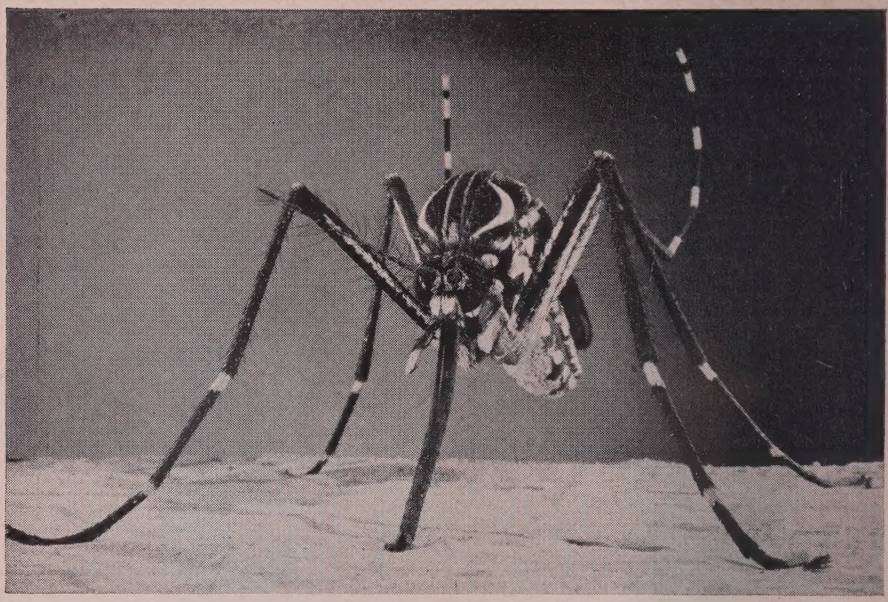
Errata

Under the section on Insect Resistance in Mosquito News change pages of Anonymous to read 355–357, in 16(4):321; also change Lividas to read Livadas in 15(1):59, 15(3):186, 16(2):178, 17(4):330 and 18(2):178.



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